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14. ABSTRACT
The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved.

15. SUBJECT TERMS
Lifestyle; Cardiovascular Disease (CVD); Prevention; Behavior; Coronary Heart Disease (CHD); Genomics; Metabolic Syndrome; PreDiabetes; Diabetes; Obesity; Stress; Sleep; CVD Risk Reduction

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INTRODUCTION

In 1998, Congress supported the need for basic and clinical research in Coronary Artery and Prostate Disease in order to reduce the incidence of these life-threatening diseases and develop more effective, more specific, and less invasive forms of therapy for patients (Public Law No. 105-262). In FY10, the Integrative Cardiac Health Project (ICHP) was identified as a cardiovascular (CV) research Center of Excellence (COE) by Health Affairs and placed into the Army Program Objective Memorandum (POM). ICHP continues its operation at the Walter Reed National Military Medical Center (WRNMMC) in Bethesda, Maryland.

Heart disease is the most common, costly, and preventable of all health problems and the Military Health System (MHS) has a large number of beneficiaries at risk for CVD. Cardiac related events make up a significant portion of non-battle disease injuries requiring evacuation from Theater jeopardizing operational effectiveness. Service members with multiple combat deployments and Wounded Warriors have increased CVD morbidity/mortality risk (2 and 3.5 fold, respectively).[1,2] Despite optimal medical therapy such as statins, there remains a residual CVD risk of approximately 69%.[3] Existing wellness programs in the Department of Defense and civilian healthcare do not adequately address CVD risk or obstacles related to healthy living which contribute to escalating CVD risk. This large gap in care can only be addressed with innovative, intensive, multi mechanistic approaches to improve CVD outcomes. There is a critical need for personalized CVD risk reduction and actionable empowerment strategies/tools to optimize health and reduce cost.

The ICHP champions the way for optimal CV Health in the MHS by conducting novel research utilizing a Systems Biology/personalized medicine design to discover and develop practical, pre-emptive and integrative approaches in order to detect and combat CVD earlier and augment traditional care before it affects the quality of life. This vision is in support of the MHS Strategic Focus and Quadruple Aim on health and wellness and complements the Army Performance Triad. ICHP's research[4] impacted the 2013 American Heart Association (AHA)/American College of Cardiology (ACC) Prevention Guidelines[5] and one of ICHP's prevention pilot models has been translated into practice complementing the US Army Surgeon General's Executive Health and Wellness Program.

ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice in an effort to achieve the following research aims:

- ❑ Improve Force Health by better understanding the CVD risk susceptibility of military specific populations as well as to understand the individual service member through leading-edge research using novel tools and technologies.
- ❑ Investigate and create transformational models of healthcare delivery through personalized CVD prevention tracks as an adjunct to traditional care.
- ❑ Refine individualized prevention strategies through statistical data modeling to define the most cost-effective and sustainable approaches in promoting cardiovascular health throughout the military lifecycle.
- ❑ Simultaneously, improve understanding of the molecular, physiological, biochemical, immunological and environmental basis of CV health and disease and to use that understanding to develop improved approaches to disease diagnosis, treatment and prevention, in line with NHLBI Strategic Plan 2008.

BODY

During this past year, the ICHP Executive Team actively engaged in finalizing the submission of our FY 2015-2019 research proposal. This submission includes ongoing ICHP research but also the design of a new ICHP randomized, controlled landmark protocol with a focus on sex-differences and biomarkers as predictors of atherosclerotic disease as well as cognitive decline and cancer. With this new science, ICHP is continuing dialogue with SysBioCube from USAMRMC, USACEHR, Fort Detrick, MD as a collaborative effort for data integration and metric analysis using their robust infrastructure in preparation for new protocol submission. SysBioCube is an integrated data warehouse and analysis platform for experimental data relating to diseases of military relevance. It brings together, under a single database environment, pathophysio-, psychological, molecular and biochemical data from mouse models of post-traumatic stress disorder and (pre-) clinical data from human PTSD patients. Dialogue is also progressing with LabCorp (formerly Liposcience) and Abbott Labs to participate in analysis of biomolecular work planned for this new science.

Additionally, on behalf of the Office of the Assistant Secretary of Defense (OASD), Health Affairs (HA) and the project sponsor, Dr. Terry Rauch, Director of Research & Development Policy & Oversight, OASD (HA), ICHP was invited to attend the Cardiovascular Care Capabilities-Based Assessment (CBA) Solutions Development Working Meeting as a COE. For over the past year, ICHP has been working with Booz Allen consultants on this project which will direct future funding (2020-2025) for Cardiovascular Care. These numerous and time intensive meetings were held in order to help identify materiel and non-materiel shortfalls and solutions in the Joint Force's ability to research, develop, and provide cardiovascular care capabilities and innovations. These meetings are ongoing.

Task #1: Complete the "*B*etter Adherence *to* *T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".
Status: Completed. Key study findings were reported in the W81XWH-11-2-0227 (FY12-14 Yr 1) Annual Report dated October 27, 2012.

Task #2: Complete the CADRe Five-Year Follow-up Protocol.
Status: Completed. Key study findings were reported in the W81XWH-11-2-0227 (FY12-14 Yr 2) Annual Report dated October 29, 2013.

Task #3: Continuation of the "Comprehensive Cardiovascular Risk Assessment and Health Program (CHP)".
Methodology
This program serves as a platform for ongoing translational research activities, a "virtual laboratory" based on scientific findings for the development of best personalized preventive practices. In other words, the platform allows ICHP to gather an expansive number of data points for each patient or subgroup of patients (eventually combined with data at a molecular level) that when leveraged will result in the creation of new tools in technology to define wellness, predict and prevent disease, and empower patients and providers to transform their healthcare.

The CHP platform has a dual purpose and is multifunctional. This platform 1) allows for multiple research protocols to be conducted as it sets the stage for recruitment, enrollment and hypothesis generation, advanced data modeling and simultaneously 2) provides a venue where research findings from these protocols can then be tested, validated and translated into application for clinical practice. Our protocols within the CHP are specifically designed to examine the effects of our military's high op tempo which predisposes our service members to accelerated atherosclerotic risk resulting from high stress, PTSD, depression, sleep insufficiency, overweight, prediabetes and prehypertension among other traditional disease risk factors.

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) along with tailored and personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists, stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up to 1000 patients may be enrolled each year. Some of the patients (such as nurses or traumatic injury patients, etc.) may be in subgroup programs because of unique needs. The CHP serves as a platform for ongoing translational research activities, a "virtual laboratory" for the development of best preventive practices and for CV educational and marketing materials.

The "Outcomes of the CPP Program" protocol provided for retrospective examination of existing data for the purpose of examination and reporting of the results of the evaluations and interventions of the CHP. With the initiation of the CHP Registry protocol (Sub Task 3.3), it was determined that this protocol no longer should remain active. Therefore, a closure report was submitted to WRNMMC Department of Research Programs (DRP) on 29 November 2015 and approved 29 January 2016. The closure documents and approval were forwarded to HRPO on 4 February 2016. Data from this "retrospective" database protocol will be included in the CHP Registry protocol registry (Sub Task 3.3).

Status:
The following electronically published manuscript was published in print:
Eliasson AH, Kashani MD, Howard RS, Vernalis MN, Modlin RE. Fatigued on Venus, sleepy on Mars-gender and racial differences in symptoms of sleep apnea. *Sleep Breath*. 2015 Mar;19(1):99-107. doi: 10.1007/s11325-014-0968-y. Epub 2014 Mar 15.

Abstract – Presented and Citation:
Kashani M, Eliasson A, Engler R, Villines T, Vernalis M. Women present with non-traditional precursors of CVD. *Preventive Cardiovascular Nurses' Association 21st Annual Symposium (PCNA)*, Anaheim, CA, 8-11 April 2015. (Poster - Selected for moderated session; received 2nd place ribbon in research competition)

Citation: Kashani M, Eliasson A, Engler R, Villines T, Vernalis M. Women present with non-traditional precursors of CVD. *J Cardiovasc Nurs* 2016;31(1):10A.

Abstract

Background: National guidelines for the evaluation of cardiovascular disease (CVD) risk provide clinicians with global recommendations without specifying differences according to sex.
Hypothesis: We hypothesized that important differences are present in the CVD risk profile of men and women which may point to the need for sex-specific assessment beyond traditional CVD risk scores.
Methods: Subjects presenting to a CVD prevention program underwent comprehensive evaluation for CVD risk including past medical history, family history, smoking exposure, perceived stress assessment with the validated Perceived Stress Scale (PSS), vital signs, anthropometrics, cardiac-relevant laboratory tests, and calculated Framingham Risk Score (FRS). Differences between men and women were assessed using unpaired t-tests.
Results: Among 300 women and 208 men (mean age 57±12 years), major differences in presentation were:

	FRS	Diagnosed Depression or Anxiety	TChol mg/dL	LDL mg/dL	Non-HDL mg/dL	LP(a) mg/dL	PSS
Women n=300	4.4	32%	197 ±43	115 ±33	134 ±41	82	23
Men n=208	10.9	18%	170 ±39	103 ±35	121 ±39	66	20
p value	<0.001	0.02	<0.001	<0.001	<0.001	0.02	0.001

TChol=total Cholesterol, LDL=low density lipoprotein, HDL=high density lipoprotein, LP(a)=lipoprotein (a)
There were no significant differences in family history of CVD (p= 0.99), smoking exposure (p=0.08), blood pressure (p=0.91 systolic, p=0.10 diastolic), BMI (p=0.66), or laboratory assessment of glucose metabolism (glucose 97.6 for men vs 95.1 for women; p=0.10; insulin 12.7 vs 12.2, p=0.57; HgA1C 5.8 vs 5.9, p=0.17).
Conclusions: Men and women present with CVD risk differently. Mean FRS was significantly lower for women despite worse lipid profiles and the presence of non-traditional precursors for CVD risk such as higher rates of depression/anxiety and perceived stress, all of which may manifest overt disease after menopause. In order to target interventions appropriately, screening approaches for CVD risk should aim to capture sex-specific vulnerabilities for CVD.

Abstract – Presented and Citation:
Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. High self-efficacy may benefit sleep quality and fatigue. Associated Professional Sleep Societies (APSS), Seattle, WA, June 2015. (poster)

Citation: Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. High self-efficacy may benefit sleep quality and fatigue. *Sleep* 2015;38:A295-A296.

Abstract

Introduction: Self-efficacy has been shown to correlate with adherence to positive health outcomes and serves as a pre-condition to promote heart healthy behaviors. Although prior studies associate self-efficacy scores with healthy diet and exercise behaviors, little is known about the association with sleep quality. Given the critical role of healthy sleep behaviors for

cardiovascular (CV) disease, we sought to correlate the role of self-efficacy with sleep quality and symptoms of fatigue.

Methods: Consecutive patients (n=89) entering a CV health promotion program completed validated questionnaires, specifically the Pittsburgh Sleep Quality Index (PSQI), fatigue visual analog scale, Rate Your Plate (RYP) diet questionnaire, an exercise question and a CV-relevant Self-Efficacy Questionnaire. In this retrospective analysis, patients were sorted into low (18-35) and high (36-45) score groups for self-efficacy. Groups were compared utilizing t-tests.

Results: Subjects scoring high for self-efficacy (n=44) were not different from those scoring low (n=45) with regard to age (56.2±11.9 vs 54.8±13.1 years, p=0.60), gender (50% vs 51% men, p=0.90), or race (p=0.66). The high self-efficacy group did show better sleep quality (PSQI=6.4±3.0 vs 7.9±4.1, p=0.05), less fatigue (3.5±2.3 vs 4.9±2.5, p=0.007), better RYP score (64.4±7.8 vs 60.0±8.5, p=0.01) and greater exercise minutes (216±131 vs 107±86, p<0.001).

Conclusions: Our findings agree with prior reports that high self-efficacy correlates with healthful diet and exercise habits. We extend this association to include better sleep quality and less fatigue. These findings suggest that efforts to increase self-efficacy may benefit both traditional measures of CV health as well as encompass non-traditional measures, such as sleep health.

Abstract – Presented and Publication Citation:

Kashani M, Eliasson A, Walizer E, Fuller C, Engler R, Villines T, Vernalis M. Early empowerment strategies boost self-efficacy to improve health outcomes. (Accepted for presentation - AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2015 Scientific Session – sessions canceled.) Presented at AHA 2015 Scientific Session, Orlando, FL. November 2015. (poster)

Citation: Kashani M, Eliasson A, Walizer E, Fuller C, Engler R, Villines T, Vernalis M. Early empowerment strategies boost self-efficacy to improve health outcomes. *Circ Cardiovasc Qual Outcomes*. 2015;8(Suppl_2):A331.

Abstract

Background: An important mechanism in improving health status in behavioral cardiovascular (CV) self-management programs is patient self-efficacy, or a patient's belief in their ability to make lifestyle changes to reach healthy goals. Little is known on when the optimal time is to incorporate self-efficacy enhancing strategies. We examined the impact of a jumpstart self-efficacy approach in the introductory phase of a multicomponent intervention on CV health outcomes.

Methods: Participants enrolled in a 12-month CV behavioral health intervention, part of a prospective registry, completed validated questionnaires focusing on the four domains of the program: nutrition (Rate-Your-Plate), exercise (minutes of continuous exercise per week), stress (Perceived Stress Scale) and sleep (Pittsburgh Sleep Quality Index); CV-relevant Self-Efficacy Questionnaire. Empowerment strategies comprised a comprehensive risk assessment report with detailed lifestyle recommendations on optimizing risk reduction and a multi-disciplinary educational workshop with interactive healthy food demonstration and stress management instruction. For the remainder of the program, patients received ongoing motivational health-coaching to achieve healthy goals in the four domains. Self-Efficacy Questionnaire was administered at baseline and after the

workshop at 6-8 weeks from baseline. All other measures at entry to and at 6-months of the program were assessed with paired t-tests.

Results: Of 88 consecutive completers of the registry, 65 subjects (77%) had a family history of premature CV disease. These high risk subjects (36 men, age 57.4 ± 12.7 years, 42 white, 15 black, 8 other) showed clinically and statistically significant improvements in all four domains as well as improvement in subjective fatigue and self-efficacy for CVD behaviors.

n=65	Self-Efficacy (of 45 points)	Diet (RYP) (of 78 points)	Activity (min/wk)	Stress (PSS) (of 56 points)	Sleep Quality (PSQI) (of 21 points)	Fatigue Visual Scale (of 10 points)
Baseline	34.3 ± 6.9	61.1 ± 8.6	156 ±114	20.7 ± 9.4	7.3 ± 3.6	4.3 ± 2.6
Outcome	40.8 ± 3.6	67.0 ± 5.9	235 ± 174	16.4 ± 8.7	4.6 ± 3.4	2.8 ± 2.2
p value	<0.001	<0.001	0.003	0.008	<0.001	<0.001

Conclusion: A comprehensive CV health intervention emphasizing empowerment strategies early in the sequence of the program improves self-efficacy leading to substantial behavioral improvements in CV health parameters. These findings are highly relevant particularly in high-risk individuals who are vulnerable to CV disease and may have the opportunity to make behavioral lifestyle modifications to lower their risk of overt disease.

Abstract – Presented and Publication Citation:

Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Villines T, Vernalis M. Prediabetes reversal using a novel comprehensive health model. Presented at ACC 2015 Scientific Session, San Diego, CA, March 2015. (poster - *Winner of Best CV Team Poster*)

Citation: Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Villines T, Vernalis M. Prediabetes reversal using a novel comprehensive health model. *J Am Coll Cardiol.* 2015;65(10_S). doi:10.1016/S0735-1097(15)61414-0.

Abstract

Introduction: Over half of prediabetics will develop frank diabetes. Prediabetes is a modifiable risk factor for cardiovascular disease (CVD) warranting preventive intervention.

Objective: We examined the impact of a multicomponent intervention on the CVD risk profile of subjects with prediabetes who successfully reversed their disease without emphasizing weight loss.

Methods: Consecutive subjects of the Integrative Cardiac Health Project Registry, a 12-month CVD Risk Reduction Program focusing on four pillars: nutrition, exercise, stress and sleep improvement, completed validated questionnaires and were categorized as prediabetic (glucose ≥ 100 mg/dL and < 140 mg/dL) or reverting prediabetes (glucose < 100 mg/dL). Diabetics were excluded from the analysis. Differences were analyzed using t-test.

Results: Of 508 subjects (56% women, mean age 53 ± 13.5 years, 61% White, 22% Black, 5% Hispanic), 107 (21%) had prediabetes with mean HgA1C 5.9% and mean glucose 108.1 mg/dL. Of prediabetics, 52 (49%) reverted to normal glucose values.

Risk Factor (n=52)	Baseline	6-month	p value
Fasting Glucose (mg/dL)	105.4 ± 6.2	92.4 ± 5.4	< 0.001
Fasting Insulin (uIU/mL)	14.5 ± 10.1	10.4 ± 7.3	0.02
Homeostatic Model Assessment	3.8 ± 2.7	2.4 ± 1.7	0.002
Total Cholesterol (mg/dL)	190.7 ± 41.1	175.1 ± 39.0	0.05
Low Density Lipoprotein (mg/dL)	115.8 ± 36.3	102.5 ± 34.7	0.06
Systolic Blood Pressure (mm Hg)	134.3 ± 15.5	127.9 ± 13.1	0.03
BMI (kg/m^2)	30.0 ± 5.7	29.0 ± 5.8	0.40
Mediterranean Diet Questionnaire (14 points)	6.8 ± 2.4	9.2 ± 2.0	0.002
Aerobic Exercise Time (min/week)	136.4 ± 139.1	192.9 ± 161.7	0.05
Perceived Stress Scale (56 points)	21.9 ± 7.4	18.7 ± 7.0	0.03
Pittsburg Sleep Quality Index (21 points)	7.0 ± 3.4	5.7 ± 3.7	0.08
Fatigue Score (10 points)	4.2 ± 1.9	3.3 ± 2.0	0.03

Conclusion: A comprehensive health program emphasizing combined improvements in nutrition, exercise, stress and sleep may help subjects with prediabetes revert to normal glucose metabolism without substantial changes in BMI. Combatting progression to diabetes with a practical lifestyle intervention lowers CVD risk and improves overall health in this vulnerable population.

<u>The following are key activities of the past year:</u>

- Total patient visits: 1577 (includes telephonic coaching calls) of which 287 visits were conducted in 4th quarter.
- ICHP Database and Platform Creation:
    - Completion of 90% of initial objectives.
    - Training of clinical staff using database process.
    - Modifications per needs of clinical staff using system.
    - Configured to launch online surveys to program participants at milestone attainment.
    - ICHP clinical guidelines incorporated to reflect latest evidence for research protocols.
    - Full launch for NPs using system for patient visits.
    - Preparation to amend potential wait-list control group for CHP.
    - Finalized data dictionary for CHP variables.
- Participated in discussions with COL Jeffrey Johnson, Director of Army Wellness at request of TSG Patricia Horoho to discuss ICHP programs and outcomes that may be scalable to complement TSG's Performance Triad. The goal was to determine which aspects of ICHP's program are exportable or could be integrated at other MTFs.
- ICHP based Cognitive Behavioral Therapy (CBT) Intervention for insomnia protocol received approval at WRNMMC, USUHS and MRMC HRPO. Recruitment started for feasibility phase (23 assessed for initial criteria; 18 excluded; 5 approached for informed consent and 2nd screening; 1 excluded; 4 randomized).
- Process for streamlining NP process developed and implemented to include comprehensive and updated clinical guidelines.
- Efforts to capture ICHP’s impact on health improvement as well as CVD risk reduction: quantitative approaches to LIFE Score (Life Impact for Empowerment) begun.
- Provided customized educational ICHP handout sent to WRNMMC Heart Failure Clinic.

- New ICHP Cookbook in development
- Process created for ZENITH study subjects to be converted to CHP patients with study closure.
- ICHP supported the following events/activities through staff participation, educational handouts/presentation, and healthy snacks, cookbooks:
  - WRNMMC Prosperity Fair
  - Wounded Warrior Prosperity Fair & Welcome Home event
  - February Health Month in collaboration with WRNMMC Cardiology Clinic
  - Four star spouses event for healthy eating and lifestyle management
  - WRNMMC Prostate Cancer Support Group
- Personnel activities impacting ICHP CHP:
  - Data Outcomes Analyst converted to Clinical Administrative Manager which included expanded duties to include supervision of front-desk personnel and functions.
  - Medical Office Administrative Assistant hired to provide both administrative front desk and medical office assistant support to CHP.
  - Loss of 2 full time and 1 part-time NP due to unexpected family illnesses and pregnancy has severely impacted ICHP's ability to see new and active patients.

Sub Task #3.1 Continuation of the "Validation of the ICHP Cardiovascular Risk Score" protocol.

Status: Completed. Key study findings were reported in the W81XWH-11-2-0227 (FY12-14 Yr 1) Annual Report dated October 27, 2012.

The following electronically published manuscript was published in print:
Kashani M, Eliasson A, Vernalis M, Bailey K, Tehaar M. A systematic approach incorporating family history improves identification of cardiovascular disease risk. *J of Cardiovasc Nurs* 2015;30(4):292-297. doi: 10.1097/JCN.0000000000000163. Epub 2014 May 20.

Sub Task #3.2: Initiate the "ZENITH (randomiZed Evaluation of a Novel comprehensIve prevention program on aTHerosclerosis progression) Trial".

Methodology

The purpose of this one-year, prospective, randomized, controlled, interventional trial is to investigate the impact of ICHP-CPP on vascular health, atherosclerosis progression and left-ventricular relaxation (diastolic function) among patients with increased lifetime CVD risk, but low short term coronary heart disease (CHD) risk (according to the Framingham Risk Score, FRS) as compared to receiving usual care (UC). Up to 170 male and female patients between 18-50 years of age with low (<10%) 10-year FRS for CHD but estimated lifetime risk (to age 95 years) of coronary death or myocardial infarction (MI) of ≥ 39% without clinically manifest CVD [MI, coronary or peripheral arterial revascularization, obstructive coronary artery disease (CAD), heart failure or cerebrovascular event] will be randomized to participation in the currently ongoing ICHP-CPP or to UC. The primary endpoint is between-group differences in the change in vascular endothelial function as measured using DTM, as reported as adjusted. Secondary endpoints are changes in measures for CIMT, cardiac diastolic function, lifetime CHD risk scores, and the ICHP CV Risk Score. It is hypothesized that patients with low-short term (Framingham 10-year CHD risk score) but high lifetime estimated risk for coronary death

or MI who participate in the ICHP-CPP will improve vascular health and reduce atherosclerosis progression when compared to those receiving usual care.

Status: In the past year, an amendment was submitted and approved on 22 July 2015 enhancing the recruitment plan to include study visibility in WRNMMC/NSA social media sites and the pre-identification of potentially eligible participants through scheduled Cardiology Clinic patients. The annual CR was submitted to WRNMMC IRB and approved on 22 Dec 2015.

Despite an enhancement in recruitment efforts, including a review of hundreds of Cardiology clinic patient records, enrollment continued to remain low during the past year. From 1 April 15 to 31 December 15, 45 patients were screened for initial inclusion resulting in 15 patients undergoing informed consent. Upon review of our enrollment rate and assessment of potential enrollment to meet the study timeline, a decision was made to stop recruitment and close the study. Study Closure documents forwarded to WRNMMC DRP on 28 January 2016 and approved 10 February 2016. Closure documents forwarded to HJF on 12 Feb 2016 for submission to HRPO.

Since initiation of study recruitment in July 2014, a total of 74 potential participants were screened, 19 enrolled and provided informed consent, 6 screened out after enrollment (5 had lifetime risk <39%, 1 was participating in a drug study), 14 randomized (7 – ICHP group; 7 – controls) and 2 withdraw (1 for unplanned relocation; 1 no longer wants to participate). There was 1 study completer (control group). One adverse event was reported during the length of this study.

Sub Task #3.3: Initiate the "Cardiovascular Health Program (CHP) Registry for the Integrative Cardiac Health Project" protocol.

Methodology:

The purpose of this study is to establish a registry to enable research on patients at risk for cardiovascular disease (CVD). All clinically derived patient-related data for subjects participating in the WRNMMC CHP will be entered into a single, secure database. At periodical intervals, assessment of the registry database will allow queries to define the impact of an integrative lifestyle change program on CVD risk over time. The ICHP Registry will utilize the ICHP database which documents demographics, responses to validated lifestyle habits questionnaires regarding exercise, diet, stress and sleep, physical examination and anthropometrics, laboratory test results, imaging, actigraphic data, clinical recommendations and consultations, participant management, and participant visits.

Patients will be offered enrollment into this study at the time of presentation if they are military health care beneficiaries and are at least 18 years of age. All participants, regardless of enrollment in the study, will receive the usual standard of care by their health care providers. Collection of medical information on ICHP subjects is accomplished through interview of patients as well as through review of medical information from other facilities providing care. Clinical data collection occurs at baseline and at the conclusion of the intervention, typically at 6 months. Additional follow up for support of the patient's gains and additional data collection occur at 12 months and annually for up to 5 years. The research component of this study will involve the analysis of clinical data collected at these intervals.

The ICHP clinical database can be queried at a single sitting with removal of all personally identifying information to perform assessments of prevalence of risks, associations of behaviors and risks, and the success of various interventions over time. Such queries take minutes to perform and can be accomplished with minimal risk to individual privacy. There is no need to maintain any linkage data as the information is harvested at a single sitting from one database requiring no marriage with external data sets.

Status:
A total of 188 active ICHP-CHP participants have been consented in a prospective fashion since December 2014; 144 of these were enrolled in this reporting period. Annual Continuing Review was approved by WRNMMC DRP on 17 March 2016; approval sent to HRPO for acknowledgement. There have been no adverse events reported for this study. Data analysis and publication of findings are ongoing.

Manuscript-Published (See Appendix A for manuscript):
Kashani M, Eliasson AH, Walizer EM, Fuller CE, Engler RJ, Villines TC, Vernalis MN. Early empowerment strategies boost self-efficacy to improve cardiovascular health behaviors. *Glob J Health Sci* 2016;8(9):322-330.

Manuscript Abstract

Background: Self-efficacy, defined as confidence in the ability to carry out behavior to achieve a desired goal, is considered to be a prerequisite for behavior change. Self-efficacy correlates with cardiovascular health although optimal timing to incorporate self-efficacy strategies is not well established. We sought to study the effect of an empowerment approach implemented in the introductory phase of a multicomponent lifestyle intervention on cardiovascular health outcomes.
Design: Prospective intervention cohort study
Methods: Patients in the Integrative Cardiac Health Project Registry, a prospective lifestyle change program for the prevention of cardiovascular disease were analyzed for behavioral changes by survey, at baseline and one year, in the domains of nutrition, exercise, stress management and sleep. Self-efficacy questionnaires were administered at baseline and after the empowerment intervention, at 8 weeks.
Results: Of 119 consecutive registry completers, 60 comprised a high self-efficacy group (scoring at or above the median of 36 points) and 59 the low self-efficacy group (scoring below median). Self-efficacy scores increased irrespective of baseline self-efficacy but the largest gains in self-efficacy occurred in patients who ranked in the lower half for self-efficacy at baseline. This lower self-efficacy group demonstrated behavioral gains that erased differences between the high and low self-efficacy groups.
Conclusions: A boost to self-efficacy early in a lifestyle intervention program produces significant improvements in behavioral outcomes. Employing empowerment in an early phase may be a critical strategy to improve self-efficacy and lower risk in individuals vulnerable to cardiovascular disease.

Abstracts –Submitted/Accepted/Presented and Publication Citation:
Engler R, Kashani M, Eliasson A, Walizer E, Fuller C, Villines T, Vernalis M. Blood pressure elevations below hypertension threshold linked to insulin resistance and dyslipidemia: An under-

recognized cardiovascular disease risk phenotype. Military Health System Research (MHSRS) Symposium 2016, 15-18 August 2016, Kissimmee, FL (submitted for oral presentation).

Abstract

Background: Cardiovascular disease (CVD) morbidity/mortality risk has been directly correlated to blood pressure (BP) levels with lower levels, even below "normal ranges", associated with reduced CVD risk. Yet current clinical guidelines only address treatment for frank hypertension (equal/over 140/90 mmHg).There is increasing interest in earlier and more precise identification of CVD risk particularly for enhanced lifestyle management interventions to prevent disease and reduce lifetime risks. Metabolic dysfunction characterized by insulin resistance predicts future risk for type 2 diabetes mellitus (T2DM) and is potentially reversible. The homeostatic model assessment (HOMA) is a calculated value that reflects hepatic insulin resistance (IR). Early preclinical diabetes with increased IR affects a large population (86 million Americans) and has gone largely unrecognized. Improving the precision of CVD risk assessments in order to guide earlier more effective intervention strategies can reduce the burden of future CVD risk complications.

Methods: Between July 2005 and July 2015, consecutive subjects entering the Integrative Cardiac Health Project (ICHP) Registry (a12-month prospective CVD Risk Reduction Program) were assessed for BP category and prevalence of metabolic risk factors by measuring anthropometrics and CVD-relevant laboratory parameters including insulin resistance by HOMA. HOMA values greater than 2.0 to 3.0 are associated with increased CVD risk in adult populations. BP was categorized as not elevated (less than 120/80), modestly elevated (between 120/80 and 140/90, also described as prehypertension) and hypertensive (equal/over 140/90). Comparisons were made between subjects with no BP elevation, modest BP elevation and hypertensives for differences in CVD risk factors using t-test analysis. These BP groups were compared for the following CVD risk parameters: fasting glucose (Gluc), hemoglobin A1C (HgbA1C), HOMA-IR, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG), body mass index (BMI), and waist circumference (WC).

Results: Of 352 subjects (56% women, mean age 53 ± 13.5years, 61% white, 22% black, 5% Hispanic), 114 (32%) had no elevation in BP,154 (44%) had modest elevation in BP and 84 (24%) were hypertensive. There were no differences between the hypertensive group and those with modest elevation in BP. There were significant differences in means (+/- SD: standard deviation) between those without elevated BP and the group with modestly elevated BP for the variables detailed: Gluc [93.9(16.7) vs 100.6(14.9), p=0.001]; HgbA1C [5.5(0.06) vs 5.7(0.06), p=0.02]; HOMA [2.89(2.6) vs 3.75(3.8), p=0.01]; HDL[60.4(17.0) vs 55.2(13.6), p=0.009]; TG [97.6(50.7) vs 115.7(66.1), p=0.012]; BMI[28.2(5.8) vs 30.5(5.5), p=0.0006]; WC [94.3(15.1) vs 102.8(14.1), p=0.0001]. There were no significant differences in LDL levels [108.5(28.7) vs 115.0(38.0), p=0.12].

Conclusion: We demonstrate that among subjects with pre-hypertension, there is a significant prevalence of insulin resistance, dyslipidemia and obesity. Modest elevations in BP may identify subjects with metabolic syndrome who may benefit from enhanced preventive interventions. Given the many military service associated confounders exacerbate CVD risk, there is a need for improved earlier diagnosis of clinical conditions that can and should be addressed to maintain optimum health of the force.

Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Targeted behavioral interventions improve disturbed sleep. Accepted for poster presentation at SLEEP 2016, Associated Professional Sleep Societies, Denver, CO, 11-15 June 2016.

Abstract

Introduction: Sleep is an established risk factor for cardiovascular disease (CVD). CVD prevention programs are an ideal setting to assess patients for disturbed sleep. For our CVD prevention program, we report the frequency of disturbed sleep and improvement of important outcomes.

Methods: At baseline, patients completed validated questionnaires: Berlin Questionnaire for sleep apnea, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and Stanford Fatigue Scale. After CVD risk assessment by a nurse practitioner, patients attended a healthy lifestyle workshop with didactics on healthy sleep practices, experiential stress reduction, and food demonstration. All patients received personalized lifestyle prescriptions. Patients with abnormal sleep surveys received customized sleep recommendations. Over 12 months, patients were coached on diet, exercise, and stress management. Validated surveys were repeated at graduation. Means and standard deviations provide descriptive statistics. Two sample t-tests measure statistical significance for changes from baseline to graduation.

Results: Of 455 consecutive program completers, 59% women, there were 61% white, 31% black, 4% Hispanic, 2% Asian, 2% other. Fiftyone patients (11%) entered the program with previously diagnosed sleep apnea. Screening for sleep apnea was positive in 217 more patients (48%) consequently referred for polysomnography. Of the remaining 187 patients (41%), 68% had poor sleep quality (mean PSQI 7.8±2.8, normal sleeper <5 points), mean sleep duration 6.6±1.2 hours, ESS 7.3±4.4, and fatigue score 3.4±2.2. Of patients with poor sleep quality (68%), PSQI improved 2.2 points, $p<0.001$; 54% improved sleep duration 30 minutes, $p=0.007$; 71% improved ESS 3 points, $p<0.001$, and 58% improved fatigue 1.2 points, $p<0.001$.

Conclusions: Our CVD prevention program provides an opportune mechanism to identify sleep disturbances. Nearly 2/3 of our population screens positive for sleep apnea and a majority of the remainder experience poor sleep quality and duration. Targeted interventions for improved sleep are effective and support CVD risk modification.

Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. Society of Behavior Medicine (SBM) 37th Annual Meeting & Scientific Session, Washington DC, 30, March 2016. (poster)

<u>Citation:</u> Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. *Ann Behav Med* 2016 Mar;50 Suppl 1:S124. doi: 10.1007/s12160-015-9766-4

<u>Abstract</u>

Background: Self-efficacy, or confidence in the ability to carry out behavior to achieve a desired goal, is considered to be a prerequisite to behavior change. Prior research has shown that efforts to improve self-efficacy correlate with greater adherence to dietary guidance and exercise prescriptions or both combined. However, the role of self-efficacy for stress management and sleep improvement has not been well studied. We sought to examine the effect of empowerment

strategies for self-efficacy on a multicomponent lifestyle intervention focusing on four behaviors: diet, exercise, stress management and sleep.
Methods: Patients in the Integrative Cardiac Health Project Registry, a prospective lifestyle change program for the prevention of cardiovascular disease were analyzed for behavioral changes using validated surveys, at baseline and one year, in the domains of nutrition, exercise, stress and sleep. Self-efficacy questionnaires (9 questions, maximum possible score 9 x 5=45 points) were administered at baseline and after the empowerment intervention, at 8 weeks. Data from baseline and one year were compared with t-tests.
Results: Of 119 consecutive program completers, 98 (82%) showed improvements in self-efficacy. Data sets were normally distributed. For all patients, self-efficacy scores increased a mean of 5.8 ± 5.1 points. There were consequent improvements in dietary adherence (61.7 ± 8.3 to 67.1 ± 6.0, R=5.8, $p<0.001$), exercise minutes (156 ± 125 to 220 ± 163, R=3.4, $p<0.001$), stress scores (20.1 ± 9.1 to 17.2 ± 8.6, R=2.6, p=0.01), sleep quality (7.1 ± 3.9 to 4.7 ± 3.5, R=4.8, $p<0.001$) and fatigue (4.3 ± 2.5 to 3.0 ± 2.2, R=4.2, $p<0.001$). These findings remained statistically significant after Bonferroni correction.
Conclusions: A boost to self-efficacy in a lifestyle intervention program produces substantial improvements in behavioral outcomes. This study validates prior reports that efforts to improve self-efficacy improves adherence to diet and exercise regimens and extends the finding to improvements in stress management and sleep.

Kashani M, Eliasson A, Walizer E, Engler R, Fuller C, Villines T, Vernalis M. Even modest elevations in blood pressure may signal dysmetabolic risk. American College of Cardiology (ACC) 2016 Scientific Session, Chicago, IL, April 2-4, 2016. (Submitted for poster – not accepted)

<u>Abstract</u>

Background: Currently, cardiovascular disease (CVD) risk may be underestimated in subjects with modest elevation in blood pressure (BP) as providers are advised to focus on treatment of frank hypertension.
Methods: Consecutive subjects entering the Integrative Cardiac Health Project Registry (a 12-month prospective CVD Risk Reduction Program) were assessed for BP category and prevalence of metabolic risk factors by measuring anthropometrics and CVD-relevant labs. BP was categorized as not elevated (≤120/80), modestly elevated (>120/80 and <140/90) and hypertensive (≥140/90). Comparisons were made between subjects with no BP elevation, modest BP elevation and hypertensives for differences in CVD risk factors using t-test.
Results: Of 352 subjects (56% women, mean age 53 ± 13.5 years, 61% white, 22% black, 5% Hispanic), 114 (32%) had no elevation in BP, 154 (44%) had modest elevation in BP and 84 (24%) were hypertensive. There were no differences between hypertensive group and group with modest elevation in BP.
Conclusion: Dyslipidemia, glucose dysmetabolism and obesity co-exist with very modest increases in BP, laying a foundation for metabolic syndrome. Left unrecognized and without targeted intervention, even patients with modest elevations in BP may be vulnerable for complications of CVD.

BP Category	BP mmHg	Glucose mg/dL	HOMA	HbA1C %	LDL mg/dL	HDL mg/dL	TG mg/dL	BMI kg/m^2	WC cm
Not Elevated n=114	112/72	93.9 ± 16.7	2.89 ± 2.6	5.5 ± 0.6	108.5 ± 28.7	60.4 ± 17.0	97.6 ± 50.7	28.2 ± 5.8	94.3 ± 15.1
Modestly Elevated n=154	128/80	100.6 ± 14.9	3.75 ± 3.8	5.7 ± 0.6	115.0 ± 38.0	55.2 ± 13.6	115.7 ± 66.1	30.5 ± 5.5	102.8 ± 14.1
p value	N/A	0.001	0.01	0.02	0.12	0.009	0.012	0.0006	0.0001
BP = blood pressure, HOMA = homeostatic model assessment, HbA1C = hemoglobin A1C, BMI = body mass index, WC = waist circumference									

Vernalis MN, Kashani M, Fuller C, Walizer E, Engler R, Eliasson A. Prescribing lifestyle behavior change reduces atherogenic triglyceride-rich lipoprotein. American Heart Association Scientific Sessions 2015, Orlando, FL, 7-11 November 2015. (submitted for poster – not accepted)

Abstract

Introduction: Triglyceride-rich lipoprotein (TRL) increases 5-year risk for cardiovascular disease (CVD) independent of LDL cholesterol (LDL-C) and is a new target for pharmacological therapy.
Objective: We sought to investigate the therapeutic impact of a lifestyle change program on reducing TRL.
Methods: A retrospective review of 568 consecutive adult graduates of the Integrative Cardiac Health Project's Registry analyzed changes in behavioral surveys, blood pressure, and a cardiac-relevant lab panel. Validated surveys measured adherence to Mediterranean Diet, number of Exercise Minutes per week, Perceived Stress Score and Pittsburgh Sleep Quality Index. Subjects were re-evaluated at the one-year mark of the lifestyle change program which was comprised of lifestyle change prescriptions and motivational coaching in these four domains. TRL was calculated by subtracting LDL-C from non-HDL cholesterol. Baseline and one-year parameters were compared using paired t-tests.
Results: Of 568 subjects (mean age 56.8 years, 234 men or 41%, 348 Caucasian, 176 African-American, 26 Latino, 18 other), 298 (53%, mean age 56.7 years, 40% men) improved their TRL.
Conclusions: A healthy lifestyle change program emphasizing behavioral strategies to improve diet, exercise, stress and sleep, produces clinically relevant improvements in blood pressure and lipids, including TRL. Prescribing lifestyle behavior change for patients at risk for CVD is an important strategy to reduce atherogenic remnant particles and other clinically relevant r isk factors concurrently.

Risk Factors (n=568)	Baseline	One-Year	Change	p value
Mediterranean Diet Score (of 10 points)	6.6±2.5	8.4±2.3	1.8	<0.0001
Exercise Minutes per Week	151±136	212±155	61	0.0002
Pittsburgh Sleep Quality Index (of 21 points)	7.1±3.9	5.5±3.6	-1.7	<0.0001
Perceived Stress Score (of 56 points)	21.1±8.6	17.8±8.1	-3.1	<0.0001

Systolic BP (mm/Hg)	128±14	125±14	-3	0.0005
Diastolic BP (mm/Hg)	80±9	78±9	-2	0.0004
Total cholesterol (mg/dL)	185.6±42.3	180.1±38.4	-5.5	0.02
Triglyceride (mg/dL)	119.2±116.5	105.2±73.3	-14.0	0.02
LDL-C (mg/dL)	109.9±33.9	105.0±32.4	-4.9	0.02
TRL (mg/dL)	18.8±21.2	16.6±12.6	-2.2	0.03

Sub Task #3.4 Collaboration on "Assessing Risk Factors for Cardiovascular Disease in Individuals with Traumatic Amputations" protocol (PI: Alison Pruziner), DPT, ATC, WRNMMC Dept of Rehab).

Methodology:

The objective of this comparative cohort study is to assess presence of known risk factors for CVD in individuals with traumatic amputations. Up to 405 participants will be enrolled and divided into three groups: no injury, traumatic orthopedic injury with amputation, traumatic orthopedic without amputation. Data will be collected at two time points, at time of consent and at a 5-year follow-up visit, and will include demographic (including diagnosis of hypertension, hyperlipidemia or diabetes mellitus) and family history, anthropometric (height, weight, waist circumference, hip circumference and body composition), biochemical (lipids, fasting blood sugar, hemoglobin A_{1c}, fasting insulin, ultra-sensitive C - reactive protein, lipoprotein (a), thyroid stimulating hormone, vitamin D, and fibrin D-dimer), blood pressure, heart rate, pulse pressure, EKG, carotid intima-medial thickness (CIMT) study, stress and sleep surveys, diet (fruit and vegetable intake, total fat and saturated fat intake), smoking history and activity measures. CVD risk will be estimated using the Integrated Cardiac Health Project (ICHP) risk assessment and the National Heart Lung and Blood Institute (NHLBI) 10-year risk estimate. It is hypothesize that: 1) Individuals with traumatic amputations (A) will have higher levels of factors that increase risk (anthropometry, biochemical markers, blood pressure, pulse pressure, CIMT, stress, poor sleep habits, saturated fat intake, smoking) and lower levels of factors that decrease risk (fruit and vegetable intake and activity) for CVD when compared to individuals without orthopedic injuries (N), and that this risk will continue to increase over the 5-year follow-up; 2) Individuals with traumatic amputations (A) will also have the same increased risk factors, as stated above, when compared to individuals with traumatic orthopedic injuries that did not result in amputation (O), and again this risk will continue to increase over the 5-year follow-up, and; 3) There will be no difference in presence of risk factors between individuals with (O) and without orthopedic injuries (N), that did not result in amputation.

Status:

Total study enrollment=54 (24 controls, 26 amputees, 4 limb salvage): 2 subjects enrolled during this reporting period. There is limited recruitment support at present. PI is seeking avenues for funding personnel position to enhance recruitment/data collection. ICHP continues to support collection of CIMT, EKG and ICHP questionnaires for risk assessment. MAP sample/PAXGene sample collection/storage began with these 2 newly enrolled subjects. Annual continuing review was approved by WRNMMC DRP on 14 Dec 2015 and forwarded to HRPO for acknowledgement.

Task #4: Follow-up data analysis and publications for the following protocols at Windber Research Institute (WRI): 1) Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal and the Sub-

<u>Study for Subjects in the Dr. Dean Ornish Program and 2) Cardiovascular Risk Assessment and Prevention Program through the Cardiovascular Risk Clinic (CRC).</u>
<u>Status:</u> Enrollment complete. Follow-up data analysis and publications are ongoing. Key study findings have been previously reported in Annual Reports for W81XWH-11-2-0227 (FY12-14).

<u>Manuscripts Published (See Appendix A):</u>
Ellsworth DL, Mamula KA, Blackburn HL, McDyer FA, Jellema GL, van Laar R, Costantino NS, Engler RJ, Vernalis MN. Importance of substantial weight loss for altering gene expression during intensive cardiovascular lifestyle modification. *Obesity (Silver Spring)* 2015 Jun;23(6):1312-9. doi: 10.1002/oby.21079. Epub 2015 May 9.

<u>Abstract</u>
Objective: To examine relationships between weight loss through changes in lifestyle and peripheral blood gene expression profiles.
Methods: A prospective nonrandomized trail was conducted over 1 year in participants undergoing intensive lifestyle modification to reverse or stabilize progression of coronary artery disease. Cardiovascular risk factors, inflammatory biomarkers, and gene expression as a function of weight loss were assessed in 89 lifestyle participants and 71 retrospectively matched controls undergoing usual care.
Results: Substantial weight loss (-15.2+3.8%) in lifestyle participants (n=33) was associated with improvement in selected cardiovascular risk factors and significant changes in peripheral blood gene expression from pre- to post-intervention: 132 unique genes showed significant expression changes (false discovery rate corrected P-value <0.05 and fold-change >1.4). Altered molecular pathways were related to immune function and inflammatory responses involving endothelial activation. In contrast, participants losing minimal weight (-3.1+2.5%, n=32) showed only minor changes in cardiovascular risk factors and markers of inflammation, and no changes in gene expression compared to non-intervention controls after 1 year.
Conclusions: Weight loss (>10%) during lifestyle modification is associated with down-regulation of genetic pathways governing interactions between circulating immune cells and the vascular endothelium and may be required to successfully reduce CVD risk.

Blackburn HL, McErlean S, Jellema GL, van Laar R, Vernalis MN, Ellsworth DL. Gene expression profiling during intensive cardiovascular lifestyle modification: Relationships with vascular function and weight loss. *Genomics Data* 2015;4:50-53. http://dx.doi.org/10.1016/j.gdata.2015.03.001

<u>Abstract</u>
Heart disease and related sequelae are a leading cause of death and healthcare expenditure throughout the world. Although many patients opt for surgical interventions, lifestyle modification programs focusing on nutrition and exercise have shown substantial health benefits and are becoming increasing popular. We conducted a year-long lifestyle modification program to mediate cardiovascular risk through traditional risk factors and to investigate how molecular changes, if present, may contribute to long-term risk reduction. Here we describe the lifestyle intervention, including clinical and molecular data collected, and provide details of the experimental methods and quality control parameters for the gene expression data generated

from participants and non-intervention controls. Our findings suggest successful and sustained modulation of gene expression through healthy lifestyle changes may have beneficial effects on vascular health that cannot be discerned from traditional risk factor profiles. The data are deposited in the Gene Expression Omnibus, series GSE46097 and GSE66175.

Manuscripts – Under Revision:

Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Kashani M, Vernalis MN. Cardiac interventions differing in lifestyle modification intensity improve insulin resistance through changes in lipoprotein profiles. (Resubmit planned to *Diabetes, Obesity, and Metabolism*)

Abstract

Aims: To determine if clinical lifestyle interventions differing in scope and intensity improve insulin resistance (IR), defined by the Lipoprotein Insulin Resistance (LP-IR) index, in patients differing in the severity of metabolic dysfunction.
Methods: Patients with diagnosed type-2 diabetes, coronary artery disease (CAD), or significant risk factors participated in one of two clinical lifestyle interventions: 1) intensive nonrandomized program with a strict vegetarian diet (N=90 subjects, 90 matched controls) or 2) moderate randomized trial following a Mediterranean-style diet (N=89 patients, 58 controls). On-treatment and intention-to-treat analyses assessed changes over one year in LP-IR, lipoprotein profiles, and metabolic risk factors in intervention patients and controls in both programs.
Results: In the on-treatment analysis, both interventions led to weight loss: [-8.9% (95% CI, -10.3 to -7.4), intensive program; -2.8% (95% CI, -3.8 to -1.9), moderate program; adjusted $p<0.001$] and a decrease in the LP-IR index [-13.3% (95% CI, -18.2 to -8.3), intensive; -8.8% (95% CI, -12.9 to -4.7), moderate; adjusted $p<0.01$] compared to respective controls. Of the six lipoprotein parameters comprising LP-IR, only large very-low-density lipoprotein (VLDL) particle concentrations decreased significantly in patients compared to controls in both programs [-26.3% (95% CI, -43.0 to -9.6), intensive; -14.2% (95% CI, -27.4 to -1.0), moderate; $p<0.05$]. Intention-to-treat analysis confirmed and strengthened the primary results.
Conclusions: Moderate lifestyle modification following a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving IR defined by the LP-IR index.

Mamula KA, Vernalis MN, Ellsworth DL. Practical considerations and potential predictors of attrition from lifestyle modification programs for cardiovascular risk reduction. (Planned submission after revision)

Abstract

Background: Identifying significant predictors of attrition from lifestyle modification programs is central to improving treatments for cardiovascular disease.
Design: Prospective clinical intervention with one year outcomes.
Methods: We examined attrition among women (n=178) and men (n=160) who enrolled in a clinical intervention designed to stabilize or reverse progression of heart disease through changes in lifestyle. Pretreatment (baseline) and initial treatment-related variables were examined separately in women and men using stepwise logistic regression to assess utility in discriminating eventual dropouts from completers.
Results: Stepwise regression models for women [$p < 0.001$, Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) = 0.772] and men ($p < 0.0001$, ROC AUC = 0.788), which

best predicted dropout or completer status, contained three common variables: body mass index at entry, dietary compliance, and education level, but neither model accurately predicted attrition. Participants reported practical reasons that caused them to discontinue participation, and these factors differed between women and men: noncompliance with the program guidelines and medical/health problems were important issues for women, while work-related conflicts were most prevalent in men.
Conclusions: Clinical trials and lifestyle programs for cardiovascular risk reduction should recognize that personal barriers to continued participation differ between women and men and must strive to accommodate all barriers in order to maximize patient retention.

Abstracts –Presented and Publication Citation:
Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Vernalis MN. Cardiac interventions differing in lifestyle modification improve insulin resistance through changes in lipoprotein profiles. American Heart Association (AHA) EPI/Lifestyle 2016 Scientific Sessions, Phoenix, AZ, March 2016. (poster)

Citation: Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Vernalis MN. Cardiac interventions differing in lifestyle modification improve insulin resistance through changes in lipoprotein profiles. *Circulation* 2016;133:AP108.

Abstract

Background: Metabolic dysfunction characterized by insulin resistance (IR) is an important risk factor for type-2 diabetes and coronary artery disease (CAD). The Lipoprotein Insulin Resistance (LP-IR) index, derived from measures of lipoprotein subclass particle concentration and size, is useful for assessing IR and identifying patients with increased diabetes/CAD risk.
Hypothesis: This study addressed the hypothesis that lifestyle modification programs differing in scope and intensity both improve IR through changes in lipoprotein profiles.
Methods: Patients with CAD or significant CAD risk factors participated in one of two clinical lifestyle interventions: 1) an intensive nonrandomized program with a strict vegetarian diet (n=90 subjects, 90 matched controls) or 2) a moderate randomized trial following a Mediterranean-style diet (n=89 participants, 58 controls). On-treatment and intention-to-treat analyses used regression modelling adjusted for CAD risk factors and lipid-lowering medication use to assess changes over one year in LP-IR, lipoprotein profiles, and CAD risk factors in intervention and control participants in both programs.
Results: Participants in the intensive lifestyle intervention had poorer baseline cardiovascular health than patients in the moderate program. In the on-treatment analysis, both lifestyle interventions led to weight loss [-8.9% (95% CI: -10.3, -7.4), intensive program; -2.8% (95% CI: -3.8, -1.9), moderate program; adjusted $p<0.001$] and a decrease in the LP-IR index [-13.3% (95% CI: -18.2, -8.3), intensive; -8.8% (95% CI: -12.9, -4.7), moderate; adjusted $p<0.01$] compared to respective controls over one year. Of the six lipoprotein parameters comprising LP-IR, only large very-low-density lipoprotein (VLDL) particle concentrations decreased significantly in patients compared to controls in both programs [-26.3% (95% CI: -43.0, -9.6), intensive; -14.2% (95% CI: -27.4, -1.0), moderate; $p<0.05$]. Intention-to-treat analysis confirmed and strengthened the primary results.

Discussion: In conclusion, moderate lifestyle modification following a Mediterranean diet is comparable to a stringent intervention with a vegetarian diet for improving IR defined by the LP-

IR index. Significant reductions in large VLDL particles may drive improvement in IR irrespective of the magnitude of lifestyle changes.

Vernalis MN, Engler RJM, Mamula KA, Blackburn HL, Kashani M, Ellsworth DL. Weight loss impact on insulin resistance: A novel lipoprotein insulin resistance index (LP-IR) identifies differing phenotypes of response to lifestyle intervention. Military Health System Research Symposium (MHSRS), Fort Lauderdale, FL, August, 2015. (podium)

Citation: None.

Abstract

Introduction: Lipoprotein Insulin Resistance Index (LP-IR) is a novel proprietary non-gender specific calculation for insulin resistance based on lipoprotein sub-particle size distribution. LP-IR is described as a reliable biomarker for progression to diabetes that reflects improvements in metabolic syndrome following dietary/lifestyle interventions with weight loss.

Objective: To compare post-diet/lifestyle intervention subjects who lost weight and decreased versus increased their LP-IR index.

Methods: Overweight/obese subjects with cardiovascular disease (CVD) or significant CVD risk factors enrolled in a 1 year intensive lifestyle intervention program including low fat (<10%) vegan diet. Risk factors, anthropometrics and biomarkers (including LP-IR, lipid profiles, etc.) associated with CVD risk were measured before and 1 year after intervention for comparison to weight loss changes. Subjects, stratified by LP-IR decrease or increase after 1 year, were compared using Wilcoxon nonparametric tests.

Results: Most participants (n=102, 49 males, 53 females) completed the program with weight-loss. Two groups were identified by LP-IR change: LP-IR score increase (25/102=24.5%); LP-IR decrease (77/102=75.5%). At baseline, there were no significant differences between these two LP-IR groups by age, BMI, systolic/diastolic BP, HDL/LDL/total cholesterol or triglycerides but mean LP-IR scores were significantly different (p=0.0019). Change in HDL-C, triglycerides, and LP-IR score after 1 year differed significantly between groups (p=0.0154, p=0.0024 and p=<0.0001, respectively).

	LP-IR Increased (N=25)			LP-IR Decreased (N=77)			Between Groups
Risk Factor	Baseline (SD)	Year 1 (SD)	% Change	Baseline (SD)	Year 1 (SD)	% Change	P-Value
BMI (kg/m2)	33.556 (7.715)	30.02 (7.409)	-10.54%	33.82 (6.73)	30.561 (6.225)	-9.64%	0.4435
Systolic BP (mm Hg)	134.16 (15.22)	126.56 (14.669)	-5.66%	137.143 (17.945)	128.182 (17.357)	-6.53%	0.7851
HDL-C (mg/dl)	48.32 (11.131)	43.96 (9.176)	-9.02%[b]	44.532 (13.5)	43.688 (11.679)	-1.90%	**0.0154**
LDL-C (mg/dl)	120.375 (32.87)	108 (29.376)	-10.28%	109.613 (39.539)	106.133 (34.511)	-3.17%	0.0555
T-CHOL (mg/dl)	206.92 (39.841)	193.8 (38.636)	-6.34%	191.26 (46.04)	179.013 (41)	-6.40%	0.7088
TG (mg/dl)	183.08 (110.671)	206.6 (116.635)	12.85%	183.701 (91.098)	147.701 (78.629)	-19.60%	**0.0024**
LP-IR	58.24 (20.001)	66.72 (21.384)	14.56%	71.662 (16.126)	55.714 (18.773)	-22.25%	**≤.0001**

Conclusion: The majority of individuals who lose weight reduce their LP-IR. However, a subgroup (25%) of patients increased their LP-IR despite weight loss. The clinical and prognostic significance of these observations require further study.

Task #6: Initiate "Exploring the Predictive Patterns of the Natural History of Pre-diabetes: Proof of Principle Study" protocol at WRNMMC (PI – COL Robert Vigersky, Diabetes Institute).
Status: Study not initiated; closed due to reallocation of funding.

*****No Funding Requested in this Award Modification for the following 2 tasks, therefore, no work is reported on this annual report.***

Task #7: Continue study entitled "Metabolic and Biomolecular Biology Study Studies in Surgical Interventions for Morbid Obesity" as a component of the Integrative Cardiac Health Program at WRI.

Task #8: Initiate the "Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal: Long-term Follow-up Sub-study at WRI.

KEY RESEARCH ACCOMPLISHMENTS

- Research Protocol Activity:
  - 6 Active: WRNMMC=3; WRI=3 (data analysis only)
  - 1 in preparation
  - Enrollment to the Cognitive Behavioral Therapy for Insomnia (ICHP CBT-I) study initiated
- ICHP Database and Platform: 90% of objectives completed
- Scientific research findings dissemination continues:
  - 5 manuscript published (2 previously published online); 2 in preparation
  - 11 abstracts submitted for peer-review
    - 7 abstracts accepted for presentation (posters=6; podium=1)
    - 6 abstracts published
    - 1 accepted for June 2016 presentation at APSS Sleep Conference
    - Poster presented at ACC Scientific Conference won "Best CV Team" award
    - Poster presented at PCNA selected for moderated session; received 2nd place ribbon in research competition
- Relevant Research Findings in this past year:
  - <u>Sleep and Self-Efficacy:</u>
    - High self-efficacy correlates with healthful diet and exercise habits.
    - Association includes better sleep quality and less fatigue.
    - Efforts to increase self-efficacy may benefit both traditional measures of CV health as well as encompass non-traditional measures, such as sleep health.
    - A boost to self-efficacy early in a lifestyle intervention program produces significant improvements in behavioral outcomes.
    - Employing empowerment strategies early in an intervention may improve self-efficacy and lower risk in individuals vulnerable to CVD empowerment.
    - Nearly 2/3 of our population screens positive for sleep apnea and a majority of the remainder experience poor sleep quality and duration; targeted interventions for improved sleep are effective and support CVD risk modification.
  - <u>Pre-Hypertension:</u>
    - Significant prevalence of insulin resistance, dyslipidemia and obesity in subjects with pre-hypertension and may benefit from enhanced preventive interventions.
    - With the many military service associated confounders exacerbate CVD risk, there is a need for improved earlier diagnosis of clinical conditions and maintain optimum health of the force.

- Triglyceride Rich Lipoprotein:
  - A healthy lifestyle change program emphasizing behavioral strategies to improve diet, exercise, stress and sleep, produces clinically relevant improvements in blood pressure and lipids, including TRL.
  - Prescribing lifestyle behavior change for patients at risk for CVD is an important strategy to reduce atherogenic remnant particles and other clinically relevant risk factors concurrently.

- Lipoprotein Insulin Resistance Index (LI-IR):
  - Majority of individuals who lose weight reduce their LP-IR, however a subgroup (25%) of patients increased their LP-IR despite weight loss.
  - The clinical and prognostic significance of these observations require further study.

- Attrition from Lifestyle Intervention Programs:
  - Clinical trials and lifestyle programs for CV risk reduction should recognize that personal barriers to continued participation differ between women and men and must strive to accommodate all barriers in order to maximize patient retention.

REPORTABLE OUTCOMES

Published Manuscripts (See Appendix A):

Blackburn HL, McErlean S, Jellema GL, van Laar R, Vernalis MN, Ellsworth DL. Gene expression profiling during intensive cardiovascular lifestyle modification: Relationships with vascular function and weight loss. *Genomics Data* 2015 Mar 12;4:50-53. http://dx.doi.org/10.1016/j.gdata.2015.03.001

Eliasson AH, Kashani MD, Howard RS, Vernalis MN, Modlin RE. Fatigued on Venus, sleepy on Mars-gender and racial differences in symptoms of sleep apnea. *Sleep Breath*. 2015 Mar;19(1):99-107. doi: 10.1007/s11325-014-0968-y. Epub 2014 Mar 15.

Ellsworth DL, Mamula KA, Blackburn HL, McDyer FA, Jellema GL, van Laar R, Costantino NS, Engler RJ, Vernalis MN. Importance of substantial weight loss for altering gene expression during intensive cardiovascular lifestyle modification. *Obesity (Silver Spring)* 2015 Jun;23(6):1312-9. doi: 10.1002/oby.21079. Epub 2015 May 9.

Kashani M, Eliasson A, Vernalis M, Bailey K, Tehaar M. A systematic approach incorporating family history improves identification of cardiovascular disease risk. *J of Cardiovasc Nurs* 2015 Jul-Aug;30(4):292-297. doi: 10.1097/JCN.0000000000000163. Epub 2014 May 20.

Kashani M, Eliasson AH, Walizer EM, Fuller CE, Engler RJ, Villines TC, Vernalis MN. Early empowerment strategies boost self-efficacy to improve cardiovascular health behaviors. *Glob J Health Sci* 2016;8(9):322-330.

Published Abstracts:

Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Villines T, Vernalis M. Prediabetes reversal using a novel comprehensive health model. *J Am Coll Cardiol*. 2015;65(10_S). doi:10.1016/S0735-1097(15)61414-0.

Kashani M, Eliasson A, Engler R, Villines T, Vernalis M. Women present with non-traditional precursors of CVD. *J Cardiovasc Nurs* 2016;31(1):10A.

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Kashani M, Eliasson A, Walizer E, Fuller C, Engler R, Villines T, Vernalis M. Early empowerment strategies boost self-efficacy to improve health outcomes. *Circ Cardiovasc Qual Outcomes*. 2015;8(Suppl_2):A331.

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Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. *Ann Behav Med* 2016 Mar;50 Suppl 1:S124. doi: 10.1007/s12160-015-9766-4

Manuscripts in Preparation:
Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Kashani M, Vernalis MN. Cardiac interventions differing in lifestyle modification intensity improve insulin resistance through changes in lipoprotein profiles. (Resubmit planned to *Diabetes, Obesity, and Metabolism*)

Mamula KA, Vernalis MN, Ellsworth DL. Practical considerations and potential predictors of attrition from lifestyle modification programs for cardiovascular risk reduction. (Planned submission after revision)

Presentation (Oral & Poster):
Kashani M, Eliasson A, Engler R, Turner E, Tschiltz N, Grunewald M, Halsey J, Villines T, Vernalis M. Prediabetes reversal using a novel comprehensive health model. Presented at ACC 2015 Scientific Session, San Diego, CA, March 2015. (poster - *Winner of Best CV Team Poster*)

Kashani M, Eliasson A, Engler R, Villines T, Vernalis M. Women present with non-traditional precursors of CVD. *Preventive Cardiovascular Nurses' Association 21st Annual Symposium (PCNA)*, Anaheim, CA, 8-11 April 2015. (Poster - Selected for moderated session; received 2nd place ribbon in research competition)

Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. High self-efficacy may benefit sleep quality and fatigue. Associated Professional Sleep Societies (APSS), Seattle, WA, June 2015. (poster)

Vernalis MN, Engler RJM, Mamula KA, Blackburn HL, Kashani M, Ellsworth DL. Weight loss impact on insulin resistance: A novel lipoprotein insulin resistance index (LP-IR) identifies differing phenotypes of response to lifestyle intervention. Military Health System Research Symposium (MHSRS), Fort Lauderdale, FL, August, 2015. (podium)

Kashani M, Eliasson A, Walizer E, Fuller C, Engler R, Villines T, Vernalis M. Early empowerment strategies boost self-efficacy to improve health outcomes. (Accepted for presentation - AHA Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke 2015 Scientific Session – sessions canceled.) Presented at AHA 2015 Scientific Session, Orlando, FL. November 2015. (poster)

Ellsworth DL, Costantino NS, Blackburn HL, Engler RJM, Vernalis MN. Cardiac interventions differing in lifestyle modification improve insulin resistance through changes in lipoprotein profiles. American Heart Association (AHA) EPI/Lifestyle 2016 Scientific Sessions, Phoenix, AZ, March 2016. (poster)

Kashani M, Eliasson A, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Strategies to boost self-efficacy promote multicomponent behavior changes. Society of Behavior Medicine (SBM) 37th Annual Meeting & Scientific Session, Washington DC, 30, March 2016. (poster)

Abstracts Accepted/Submitted:
Vernalis MN, Kashani M, Fuller C, Walizer E, Engler R, Eliasson A. Prescribing lifestyle behavior change reduces atherogenic triglyceride-rich lipoprotein. American Heart Association

Scientific Sessions 2015, Orlando, FL, 7-11 November 2015. (Submitted for poster – not accepted)

Kashani M, Eliasson A, Walizer E, Engler R, Fuller C, Villines T, Vernalis M. Even modest elevations in blood pressure may signal dysmetabolic risk. American College of Cardiology (ACC) 2016 Scientific Session, Chicago, IL, April 2-4, 2016. (Submitted for poster – not accepted)

Eliasson A, Kashani M, Fuller C, Walizer E, Engler R, Villines T, Vernalis M. Targeted behavioral interventions improve disturbed sleep. SLEEP 2016, Associated Professional Sleep Societies, Denver, CO, 11-15 June 2016. (Accepted for poster presentation)

Engler R, Kashani M, Eliasson A, Walizer E, Fuller C, Villines T, Vernalis M. Blood pressure elevations below hypertension threshold linked to insulin resistance and dyslipidemia: An under-recognized cardiovascular disease risk phenotype. Military Health System Research (MHSRS) Symposium 2016, 15-18 August 2016, Kissimmee, FL. (Submitted for oral presentation).

CONCLUSION

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could nearly eliminate the development of CHD and substantially decrease CHD morbidity and mortality. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. We also have shown that molecular change occurs during lifestyle modification, but this change may be transient and may be dependent on maintaining a healthy lifestyle. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

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APPENDIX A

PUBLISHED MANUSCRIPTS

Genomics Data 4 (2015) 50–53

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Data in Brief

Gene expression profiling during intensive cardiovascular lifestyle modification: Relationships with vascular function and weight loss

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ABSTRACT

Heart disease and related sequelae are a leading cause of death and healthcare expenditure throughout the world. Although many patients opt for surgical interventions, lifestyle modification programs focusing on nutrition and exercise have shown substantial health benefits and are becoming increasing popular. We conducted a year-long lifestyle modification program to mediate cardiovascular risk through traditional risk factors and to investigate how molecular changes, if present, may contribute to long-term risk reduction. Here we describe the lifestyle intervention, including clinical and molecular data collected, and provide details of the experimental methods and quality control parameters for the gene expression data generated from participants and non-intervention controls. Our findings suggest successful and sustained modulation of gene expression through healthy lifestyle changes may have beneficial effects on vascular health that cannot be discerned from traditional risk factor profiles. The data are deposited in the Gene Expression Omnibus, series GSE46097 and GSE66175.

Specifications	
Organism/cell line/tissue	*Homo sapiens*/whole blood
Sex	Male and female
Sequencer or array type	Affymetrix GeneChip HG-U133A 2.0 arrays
Data format	Raw data: CEL/TAR files, Normalized data: SOFT, MINiML, TXT
Experimental factors	Clinical: Standard demographic and clinical information, physiological and biochemical assessment; Molecular: RNA isolated from PAXgene™ tubes, globin reduction treatment of RNA, standard Affymetrix expression analysis, transcript validation by qRT-PCR
Experimental features	Intensive lifestyle modification to stabilize or reverse progression of heart disease over 1 year; participants and retrospectively matched controls with CAD or 2+ risk factors; group comparisons; risk factor correlations with gene expression; functional enrichment and pathways analysis; medication influence
Consent	All patients provided a written informed consent before participation. The study protocol (Pro00009375) was approved by the Chesapeake Institutional Review Board (https://www.chesapeakeirb.com/).
Sample source location	Windber, Pennsylvania, USA

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E-mail address: d.ellsworth@wriwindber.org (D.L. Ellsworth).

Direct link to deposited data

The study is registered as NCT01805492 at ClinicalTrials.gov. Expression data were deposited in the Gene Expression Omnibus (GEO) under series accession numbers GSE46097 and GSE66175 and are available here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46097 and http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66175.

Experimental design, materials and methods

Objectives

The main objectives of this project were to 1) characterize longitudinal changes in gene expression in peripheral blood during an intensive cardiovascular lifestyle intervention, and 2) identify associations between gene expression profiles and changes in quantitative heart disease risk factors during the intervention. Our goal was to provide a global view of molecular changes associated with drastic lifestyle modification designed to stabilize or reverse heart disease and ascertain molecular pathways that are important in the development of coronary atherosclerosis.

http://dx.doi.org/10.1016/j.gdata.2015.03.001

Study participants

Inclusion criteria for participation included: 1) adult 21+ years of age, 2) mentally competent to provide informed consent and accurately report adherence, 3) physician diagnosis of coronary artery disease (CAD), which included stable angina, angioplasty, >50% luminal narrowing on coronary angiogram, acute myocardial infarction, bypass surgery, or stent placement, or 2+ CAD risk factors such as obesity (BMI >30), hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), high total cholesterol (>200 mg/dL), diabetes, or family history of heart disease in parents or siblings, 4) approval from personal physician, 5) desire to pursue intensive lifestyle modification as an alternative to, or in conjunction with, standard therapy and motivation to follow the program guidelines for one year, and 6) successful abstinence from smoking for at least three months prior to and during enrollment.

Exclusion criteria were: 1) <21 years of age, 2) presence of unstable coronary syndromes, refractory congestive heart failure, uncontrolled arrhythmia, or high-grade uncorrected cardiac conduction abnormalities, 3) significant left main stenosis (>50%) and ejection fraction <35% in patients who did not have revascularization or were not candidates for revascularization, 4) hypotensive response to exercise, 5) known history of autoimmune disease or systemic/chronic disease requiring chemotherapy or long term treatment, 6) history of substance abuse (including alcohol) without self-certification of abstinence for at least three months, and 7) physical disabilities or medical conditions that would preclude program adherence.

Non-intervention controls were recruited prospectively and matched to program participants based on age (within ±5 years), gender, and disease status (presence of CAD or diabetes mellitus) [1]. Control subjects received only standard care from their primary care physician, did not receive any advice, counseling, or information regarding healthy lifestyle behaviors, and did not participate in any component of the lifestyle intervention.

Intervention

A prospective nonrandomized trial based on the Multicenter Lifestyle Demonstration Project was designed to stabilize or reverse progression of heart disease through comprehensive changes in lifestyle [2]. Participants were recruited by referral from physicians and through advertisements in the media. The lifestyle intervention consisted of a low-fat vegetarian diet (<10% of calories from fat), 180 min/week of moderate aerobic exercise, 1 h of stress management each day, and weekly group support sessions. The year-long program was divided into 2 stages, consisting of an intensive 3-month intervention during which participants were taught to adopt and strictly adhere to the program guidelines followed by a 9-month primarily self-directed maintenance phase.

Clinical information was collected by review of medical records, standard questionnaires, and physical examinations at the baseline, 3-month, and 1-year time points. Demographic and lifestyle factors included the following: age, gender, ethnicity, family history of disease, medication use, various psychometric parameters, and daily caloric intake. Clinical information encompassed: height and weight, systolic and diastolic blood pressure, general endurance, standard lipid panel, lipoprotein profiles, and plasma biomarkers including C-reactive protein, ultra-sensitive insulin, and leptin.

This research was conducted in accordance with the Code of Ethics of the World Medical Association. Participants and controls volunteered to participate in the research study and provided a written informed consent. All research activities were governed by the United States Army Medical Research and Materiel Command (MRMC)/Telemedicine and Advanced Technology Research Center (TATRC) and the Henry M. Jackson Foundation for the Advancement of Military Medicine. Our data reporting followed recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group [3].

Blood collection, RNA isolation, and microarray analysis

Peripheral blood was collected from participants and controls at each time point using the PreAnalytiX PAXgene™ Blood RNA System (Qiagen, Valencia, CA). Blood was placed at room temperature for 4–24 h and frozen at −80 °C. PAXgene™ tubes were thawed overnight at room temperature and RNA isolation was performed using the PAXgene™ blood RNA Kit. Globin mRNA transcripts were depleted from a portion of the total RNA using the GLOBINclear™-Human Kit (Life Technologies, Carlsbad, CA). RNA quality was assessed with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and quantity was measured with the Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA). One microgram of globin-depleted RNA was then amplified using the MessageAmp™ II aRNA Amplification System (Life Technologies). Resulting double-stranded cDNA was purified, amplified, and labeled with biotin-11-UTP. Labeled aRNA (15 μg) was subsequently fragmented and hybridized to GeneChip® Human Genome U133A 2.0 arrays (Affymetrix, Santa Clara, CA) and scanned on a GeneChip® Scanner 3000 using standard Affymetrix protocols. All 3 time points for each participant/control were processed together to minimize technical artifact. Further details of RNA isolation and gene expression analysis are available in the Data Supplement of Ellsworth et al. [4].

Quality control analysis

All CEL files (n = 480) were subjected to pre-processing using the Robust Multichip Algorithm (RMA). Probe set intensities were obtained by RMA background correction, quantile normalization, median polish summarization, and $\log_2$ transformation. To assess data integrity, evaluate assay performance, and ensure suitability for analysis, the processed intensity data was subjected to standard GeneChip® quality control parameters: background intensity, raw noise (Q) values, percent present calls, scaling factors, and GAPDH 3′/5′ ratio, and Actin 3′/5′ ratio. In addition, the following QC assessments were conducted: array image analysis to identify artifacts on the array surface, distribution analysis to assess the spread of the data relative to the full probe set, and principal component analysis to summarize overall variance.

Arrays included in the final dataset passed the recommended GeneChip® quality control assessments. The RMA normalized $\log_2$ intensity plot showed consistency of individual arrays relative to the entire dataset (Fig. 1). Principal Component Analysis identified limited variability attributable to laboratory procedures across all arrays (Fig. 2). Comparable percent present values (median = 59.2%, range 48.1–64.8%), assessed using the mean absolute deviation, were observed for all samples (Fig. 3).

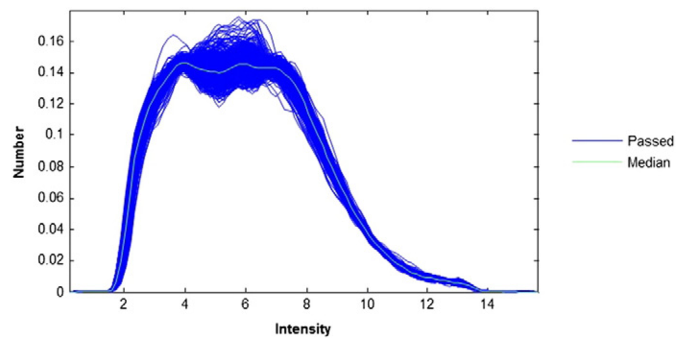

Fig. 1. Intensity graph showing the RMA normalized $\log_2$ intensity for each array. The median intensity curve is highlighted in green.

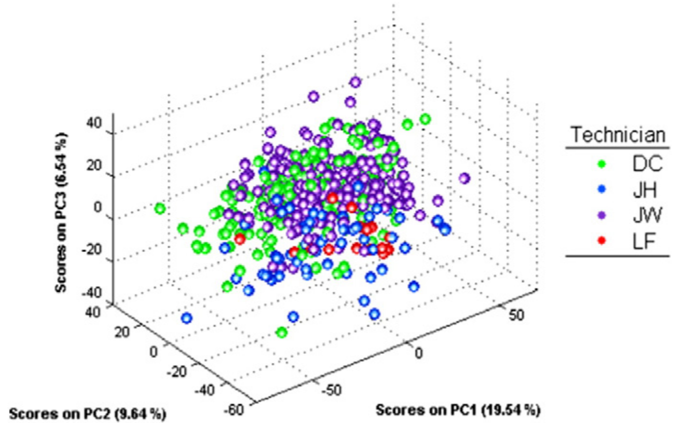

Fig. 2. Three-dimensional scatter plot representing a Principal Component Analysis of all expression arrays colored by laboratory technician.

Duplicate blood samples were collected from 7 randomly-selected participants at each examination and applied to U133A 2.0 arrays as outlined above to evaluate consistency of gene expression among duplicate assays. The average Pearson correlation for the pair-wise comparisons of RMA normalized intensities was 0.992 ± 0.006 (range 0.969–0.996) indicating high repeatability of the microarray data. Paired t-tests identified 9 genes that were differentially expressed between duplicate samples based on a false discovery rate (FDR) adjusted p-value < 0.05 and thus were excluded from further analysis: CKLF-like MARVEL transmembrane domain containing 6 (CMTM6); dehydrogenase/reductase (SDR family) member 9 (DHRS9); guanine nucleotide binding protein (G protein), α11 (GNA11); kelch-like 18 (KLHL18); kinesin family member 1A (KIF1A); mitogen-activated protein kinase 1 interacting protein 1-like (MAPK1IP1L); nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1); transportin 1 (TNPO1); and vesicle-associated membrane protein 1 (synaptobrevin 1) (VAMP1).

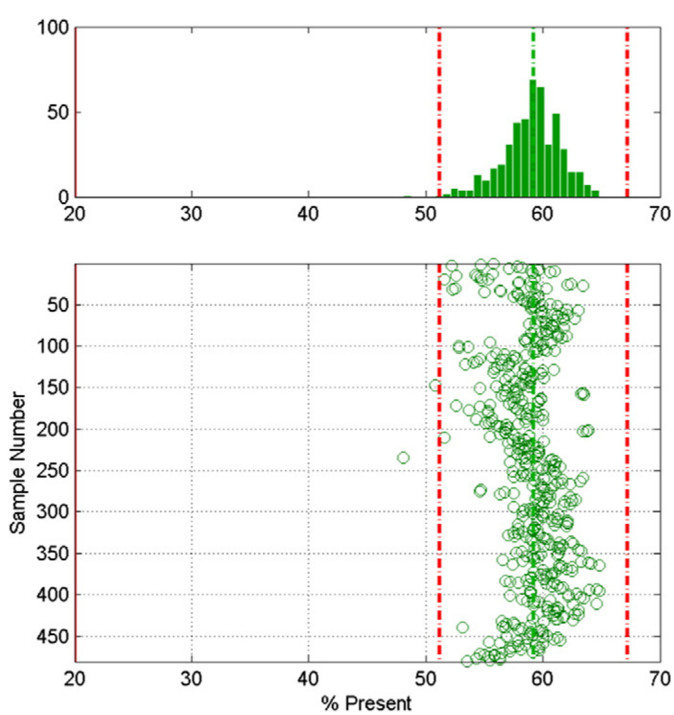

Fig. 3. Histogram (top panel) and scatter plot (bottom panel) showing the percentage of probes on each array yielding detectable expression (percent present calls). The median of the percent present calls is represented by the dashed green line and statistical limits [$\pm 3.5 \times$ STD (mean absolute deviation)] by the dashed red lines.

Basic analyses

Our research summarizing changes in physiological [5] and psychosocial risk factors [6], lipoprotein subclass profiles [7], and plasma biomarkers of cardiometabolic risk [8] during the intensive lifestyle intervention has been published previously. In all studies, lifestyle participants experienced dramatic changes in dietary measures and significant improvement in a variety of cardiovascular risk factors compared to controls.

For studies of gene expression, we first selected a subset of 63 participants and 63 matched controls to examine the impact of the lifestyle program on individual gene expression profiles and regulatory pathways important to cardiovascular health. Using ANOVA with FDR correction for multiple testing, we identified 143 genes that were differentially-expressed from baseline to 1 year in lifestyle participants but observed little change in gene expression among controls. Lifestyle modification reduced the expression of proinflammatory genes associated with neutrophil activation and molecular pathways that are important to vascular function [4].

Many genes with the largest fold-changes were significantly correlated with body mass index (BMI) throughout the lifestyle program; therefore, we next examined relationships between weight loss and changes in leukocyte gene expression in 89 lifestyle participants and 71 matched controls. Substantial weight loss ($-15.2 \pm 3.8\%$) during the program was associated with improvement in selected cardiovascular risk factors, significant changes in gene expression, and alterations in molecular pathways related to immune function and endothelial activation. Conversely, participants losing minimal weight ($-3.1 \pm 2.5\%$) showed only minor changes in risk factors, markers of inflammation, and gene expression compared to non-intervention controls [9].

Discussion

We describe detailed technical and analytical methods for a dataset of 480 Affymetrix GeneChip® U133A 2.0 arrays from 89 participants in an intensive year-long cardiovascular lifestyle intervention and 71 prospectively matched controls. To our knowledge, this is the largest gene expression dataset on participants in a cardiovascular risk reduction program. We believe this data will be of great value to future investigations examining molecular changes that occur in patients embracing healthy lifestyles in addition to the importance of lifestyle in ameliorating cardiovascular risk.

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Sleep Breath (2015) 19:99–107
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ORIGINAL ARTICLE

Fatigued on Venus, sleepy on Mars—gender and racial differences in symptoms of sleep apnea

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Abstract
Objective Clinical guidelines for the care of obstructive sleep apnea (OSA) recommend evaluation of daytime sleepiness but do not specify evaluation of fatigue. We studied how subjects with and without OSA experience fatigue and sleepiness, examining the role of gender and race.
Design, setting, patients Consecutive subjects entering our heart health registry completed validated questionnaires including Berlin Questionnaire for OSA, Fatigue Scale, and Epworth Sleepiness Scale. Data analysis was performed only with Whites and Blacks as there were too few subjects of other races for comparison.
Results Of 384 consecutive subjects, including 218 women (57 %), there were 230 Whites (60 %) and 154 Blacks (40 %), with average age of 55.9±12.8 years. Berlin Questionnaires identified 221 subjects (58 %) as having high likelihood for OSA. Fatigue was much more common in women (75 %) than in men (46 %) with OSA ($p<0.001$), while frequency of fatigue was similar in women (30 %) and men (29 %) without OSA ($p=0.86$). In multivariate analysis, men with OSA were sleepier than women; Black men with OSA had higher Epworth scores (mean±SD, 12.8±5.2) compared to White men (10.6±5.3), White women (10.0±4.5), and Black women (10.5±5.2), $p=0.05$. These gender differences were not related to the effects of age, body mass index, perceived stress, sleep duration, or thyroid function.
Conclusions Women report fatigue more commonly with OSA than men. Men experience sleepiness more commonly with OSA than women. The findings suggest that evaluation of sleep disorders must include an assessment of fatigue in addition to sleepiness to capture the experience of women.

Keywords Sleepiness · Fatigue · Obstructive sleep apnea syndrome · Sleep apnea

Abbreviations

BMI	Body mass index
CMS	Centers for Medicare and Medicaid Services
CPAP	Continuous positive airway pressure
EDS	Excessive daytime somnolence
ESS	Epworth sleepiness scale
ICHP	Integrative Cardiac Health Project
IRB	Institutional Review Board
OSA	Obstructive sleep apnea
OSAS	Obstructive sleep apnea syndrome
PSS	Perceived stress scale
SD	Standard deviation
TSH	Thyroid-stimulating hormone

Introduction

Obstructive sleep apnea (OSA) is an important disorder because of its high prevalence [1], the constellation of comorbidities associated with the disorder [2], and the substantial symptoms that OSA may cause [3]. OSA is labeled

Presentation at a Conference Portions of these data were presented as an abstract in poster format at the American Thoracic Society Meeting 18 to 23 May 2012 in San Francisco, California.

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obstructive sleep apnea syndrome (OSAS) when adequate numbers of apneas and hypopneas are accompanied by symptoms such as excessive daytime sleepiness (EDS), fatigue, inattentiveness, moodiness, or morning headaches [4].

In addition to their role in diagnosis of the syndrome, symptoms also serve as important indicators to track response to therapy. A recently published clinical guideline for evaluation and management of OSA [5] endorses the evaluation of sleepiness with the Epworth Sleepiness Scale (ESS) [6] but does not suggest an assessment of fatigue. Other recently published research demonstrates that the ESS is commonly used to evaluate OSA-associated symptoms without incorporation of a scale to measure fatigue [7, 8]. However, subjects with OSA more frequently use terms such as fatigue, tiredness, or lack of energy rather than sleepiness to characterize their symptoms pointing to a lack of connection between the questions asked to elicit symptoms and the experience of symptoms by patients with OSA [9, 10].

Furthermore, symptoms of OSAS are not experienced to the same degree by patients with similar severities of OSA as measured by apnea–hypopnea index or oxygen desaturation [9, 11]. The range and severity of symptoms caused by the sleep disruption of OSA appear to be trait-like qualities for an individual patient [12, 13] and differ markedly among individuals [11]. Substantial data support the contention that sleepiness and fatigue are independent manifestations of sleep disorders and that patients may report one or the other, both or neither while carrying the same objective diagnosis of OSA [9, 10, 14, 15]. While prior research has examined gender differences in symptoms of OSAS [9, 15], we sought to broaden our understanding of the experience of sleepiness and fatigue in subjects with and without OSA with special attention to the roles of gender and race. Such an evaluation has not been previously undertaken.

Methods

This study was conducted in accordance with the amended Declaration of Helsinki and with the approval of the Institutional Review Board (IRB) at the Walter Reed National Military Medical Center in Bethesda, Maryland, which granted approval for the protocol designated #372910. The study design is an analysis of data prospectively collected on consecutive patients enrolled in the Integrative Cardiac Health Project (ICHP) Registry. The ICHP Registry is a cardiovascular disease prevention program operating in a research Center of Excellence for the United States Department of Defense. Because the Registry database could be de-identified before data analysis, an exempt protocol was approved by the IRB (#20012) to perform a secondary analysis on the Registry data and patient consent was not required for the purpose of this analysis.

Patients are self-referred or referred to the ICHP Registry by a health care provider to improve habits of diet, exercise, sleep, and stress management. ICHP is accessible to military health care beneficiaries including active duty service members, retirees, and civilian dependents. The program therefore enrolls a broad spectrum of subjects including a variety of races and ethnic backgrounds, both genders, and a range of ages from 18 to 90 years. The typical patient entering the program is found to have two to four risk factors for cardiovascular disease.

Upon entry, subjects are asked to complete a series of questionnaires (described in detail below) to gather information on demographics, current symptoms, and lifestyle habits. Among the questionnaires are validated surveys to assess sleep behaviors, sleep quality, and daytime symptoms. Data from the questionnaires are reviewed during a medical interview with a nurse practitioner who performs a physical examination with anthropomorphic measures. Patients also submit blood for laboratory tests including a thyroid function panel.

Berlin questionnaire

Of questionnaires available to screen patients for sleep apnea, the Berlin Questionnaire is one of the most commonly utilized and best validated [16]. Permission was granted by the copyright owner to use the questionnaire for this study. As measured by the questionnaire, patients with persistent and frequent signs and symptoms are considered to be at high risk for sleep apnea. Questions about symptoms demonstrated internal consistency (Cronbach correlations, 0.86 to 0.92). With a positive Berlin questionnaire, sleep apnea was predicted with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.89, and a likelihood ratio of 3.79.

Fatigue Scale

The Fatigue Scale is borrowed from the Stanford Patient Education Research Center [17]. The Stanford web site stipulates that the scale is free to use without permission. The Fatigue Scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. The Fatigue Scale was tested on 122 subjects deriving a data set with mean score of 4.89±2.71 points. Subjects who circle 5 to 6 express mild fatigue, 7 to 8 moderate fatigue, and 9 to 10 severe fatigue.

Epworth sleepiness scale

The ESS is the most widely used tool to estimate the subjective symptom of daytime sleepiness [18]. Dr. Johns permits use of the ESS by individual people (including clinicians and researchers) free of charge. Subjects are asked to use a scale of 0 to 3 to estimate their likelihood of dozing in eight different

situations in recent weeks. The individual scores are summed and possible scores range from 0 to 24. Sleepy subjects score 11 or higher and sleepiness can be categorized by scores: 11 to 14, mild sleepiness; 15 to 19, moderate sleepiness; and 20 to 24, severe sleepiness.

Perceived stress scale

The perceived stress scale (PSS) is one of the most widely accepted measures of stress [19]. Dr. Cohen's web site, where a copy of the PSS is provided, states that permission for use of the scale is not necessary when use is for academic research or educational purposes. This validated 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0 to 56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Score in the low 20s reveal moderate stress levels while scores approaching 30 are substantial and concerning.

Statistical analysis

Continuous data that were normally distributed (as determined by the Shapiro–Wilk test) are presented using means with standard deviations (mean±SD): Univariate comparisons are made using the two-sample t test or analysis of variance. Categorical data are presented as counts with proportions and groups are compared using Fisher's exact test. Sleepiness was defined as a score on the ESS of 11 or higher, and fatigue was defined as a score on the Fatigue Scale of 5 or higher.

To adjust for confounding variables, multivariable linear regression was used with either the Fatigue Scale or ESS as the dependent variable and independent variables to include gender, race, age, body mass index (BMI), PSS, thyroid-stimulating hormone (TSH), and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariable models [20]. Data were analyzed using IBM SPSS Statistics for Windows (v. 21.0. IBM Corp. Armonk, NY).

Results

The ICHP Registry enrolled 446 participants. The mean age± standard deviation (SD) of the participants was 55.0± 12.8 years consistent with a spectrum of lifestyles from actively working to semi-retired to fully retired adults. Of the 446 consecutive subjects, 249 women (56 %), there were 234 Whites, 155 Blacks, 13 Hispanics, 2 Asians, and 42 others. Because there were so few participants represented by racial categories other than Whites and Blacks, the other races were not considered further, leaving 389 subjects. Five subjects did not have Epworth or Fatigue Scale data leaving 384 evaluable subjects with an average age of 55.9±12.8 years and including 218 women (57 %).

Fatigue was found in 181 subjects (48 %) and sleepiness in 160 subjects (42 %). The proportion of subjects reporting neither fatigue nor sleepiness, fatigue only, sleepiness only, or both fatigue and sleepiness are shown in Table 1 by race and gender. Women had higher Fatigue Scale scores (Table 2, $p=0.02$), and complained more frequently of fatigue (115 of 215, 53 %) than men (66 of 165, 40 %), while men had significantly higher Epworth scores (Table 3, $p=0.02$), and complained more frequently of sleepiness (77 of 166, 46 %) compared to women (83 of 218, 38 %).

Berlin Questionnaires identified 219 subjects (58 %) as having high likelihood for OSA. There was no difference in thyroid function between subjects with and without a positive Berlin score (mean±SD in each group was 2.2±1.4, $p=0.61$). Symptoms of fatigue and sleepiness are presented in Figs. 1 and 2. Fatigue associated with OSA is more commonly experienced by women than by men, $p<0.001$ (Table 2 and Fig. 1). Sleepiness in association with OSA is more frequently experienced by men, particularly Black men, than by all other categories, $p=0.05$ (Table 3 and Fig. 2).

Univariate analysis of Fatigue Scale scores (Table 2) demonstrates significantly higher scores in younger age groups ($p<0.001$), and in subjects with positive Berlin score ($p<0.001$), higher perceived stress scores ($p<0.001$), and shorter sleep duration ($p<0.001$). Notably, Fatigue Scale scores were not different according to TSH, nor were they different according to BMI categories after factoring in presence of OSA (Table 2).

Univariate analysis of ESS scores (Table 3) show higher scores in younger age categories ($p<0.001$), and in subjects with positive Berlin scores ($p<0.001$), higher perceived stress scores ($p<0.001$), and shorter sleep duration ($p<0.001$). ESS scores were not different according to TSH, nor were they different according to BMI categories after factoring in presence of OSA (Table 3).

To control for confounding demographic and clinical characteristics, multivariable linear regression was used to examine both fatigue and sleepiness. With the Fatigue Scale score as the dependent variable, age and perceived stress score both significantly correlated with fatigue in subjects without OSA. Younger age and higher stress were associated with more fatigue. However, among subjects with OSA, gender was also

Table 1 Symptoms by gender and race

Subject descriptors	All subjects[a] (n=380)	Black women (n=89)	White women (n=126)	Black men (n=63)	White men (n=102)	p value
Age (years)	56.0±12.8	52.9±12.0	56.9±12.0	52.1±13.6	59.9±12.9	<0.001
BMI (kg/m^2)	30.7±5.4	32.5±5.8	29.2±5.3	31.2±4.6	30.7±5.1	<0.001
Not fatigued, not sleepy	141 (37 %)	23 (26 %)	54 (43 %)	25 (40 %)	39 (38 %)	0.007
Fatigued only	81 (21 %)	28 (31 %)	29 (23 %)	6 (9 %)	18 (18 %)	
Sleepy only	58 (15 %)	9 (10 %)	14 (11 %)	15 (24 %)	20 (20 %)	
Both fatigued and sleepy	100 (26 %)	29 (33 %)	29 (23 %)	17 (27 %)	25 (24 %)	

Age, BMI, and the proportion of subjects reporting neither fatigue nor sleepiness, fatigue only, sleepiness only, or both fatigue and sleepiness are shown by race and gender. For age and BMI, comparisons between groups are made using analysis of variance. For the categorical variables of fatigue and sleepiness, comparisons between groups are made using Fisher's exact test. Fatigue was defined as a score on the Fatigue Scale of 5 or higher, and sleepiness was defined as a score on the Epworth Sleepiness Scale of 11 or higher

[a] Three hundred eighty of the 384 subjects had both Epworth and fatigue data

significantly associated with fatigue, with women reporting higher fatigue scores compared to men (Table 4).

Multiple linear regression using ESS score as the dependent variable showed that the independent variable of sleep duration was significantly associated with sleepiness among subjects without OSA, with longer sleep times associated with lower ESS scores. However, among subjects with OSA, PSS and gender were significantly associated with ESS scores. Increases in perceived stress were associated with higher levels of sleepiness. Since female gender was the reference group in the model, the positive beta coefficient for gender indicates a greater degree of sleepiness in men compared to women (Table 5).

Table 2 Fatigue scale data compared for subjects with and without OSA

Fatigue scale		Total			No OSA			OSA		
		n	mean±SD	p value	n	mean±SD	p value	n	mean±SD	p value
All subjects		380	4.4±2.4		161	3.4±2.2		219	5.1±2.3	
Gender	Females	215	4.7±2.5	0.022	105	3.5±2.3	0.58	110	5.8±2.2	<0.001
	Males	165	4.1±2.3		56	3.3±2.1		109	4.5±2.3	
Race	Black	152	4.8±2.4	0.028	56	3.6±2.3	0.54	96	5.4±2.3	0.1
	White	228	4.2±2.4		105	3.4±2.1		123	4.9±2.4	
Gender × race	Black females	89	5.3±2.5	0.002	33	4.0±2.4	0.26	56	6.0±2.3	<0.001
	White females	126	4.2±2.4		72	3.3±2.1		54	5.5±2.2	
	Black males	63	4.0±2.1		23	3.0±1.9		40	4.6±2.0	
	White males	102	4.1±2.4		33	3.6±2.2		69	4.4±2.4	
Age (years)	<50	106	5.6±2.0	<0.001	39	4.6±2.0	<0.001	67	6.2±1.8	<0.001
	50-59	131	4.5±2.5		48	3.6±2.3		83	5.0±2.5	
	60+	143	3.5±2.2		74	2.8±1.9		69	4.3±2.3	
BMI	Normal	51	4.4±2.7	0.02	31	3.5±2.5	0.61	20	5.8±2.6	0.47
	Overweight	129	4.0±2.4		78	3.3±2.2		51	5.0±2.5	
	Obese	200	4.7±2.3		52	3.6±2.1		148	4.3±2.3	
Berlin questionnaire	Normal	161	3.4±2.2	<0.001	161	3.4±2.2				
	OSA	219	5.1±2.4					219	5.1±2.4	
TSH (mU/L)	<4.5	361	4.4±2.4	0.29	154	3.4±2.2	0.3	207	5.1±2.4	0.68
	4.5 +	19	5.0±2.2		7	4.3±1.6		12	5.4±2.4	
PSS (of 56 points)	<21	176	3.4±2.3	<0.001	92	2.7±2.0	<0.001	84	4.1±2.4	<0.001
	21+	200	5.3±2.2		69	4.4±2.1		131	5.8±2.1	
Sleep duration (h)	<6	120	5.4±2.3	<0.001	37	4.3±2.5	0.005	83	5.8±2.1	<0.001
	6+	257	4.0±2.4		122	3.2±2.1		135	4.7±2.4	

Fatigue scale data are presented according to various categories listed on the left column of the table. Comparisons between groups are made using the two-sample t test or analysis of variance

Table 3 Epworth score data compared for subjects with and without OSA

Epworth score		Total			No OSA			OSA		
		n	mean±SD	*p* value	*n*	mean±SD	*p* value	*n*	mean±SD	*p* value
All subjects		384	9.4±5.2		163	7.5±4.7		221	10.9±5.1	
Gender	Females	218	8.9±5.0	0.024	106	7.4±4.8	0.87	112	10.3±4.9	0.096
	Males	166	10.1±5.3		57	7.5±4.4		109	11.4±5.3	
Race	Black	154	10.4±5.4	0.002	57	8.7±5.1	0.015	97	11.5±5.3	0.11
	White	230	8.7±4.9		106	6.8±4.3		124	10.4±4.9	
Gender × race	Black females	91	9.9±5.2	0.001	34	8.9±5.0	0.11	57	10.5±5.2	0.05
	White females	127	8.2±4.8		72	6.7±4.6		55	10.0±4.5	
	Black males	63	11.2±5.6		23	8.4±5.4		40	12.8±5.2	
	White males	103	9.4±5.1		34	7.0±3.5		69	10.6±5.3	
Age (years)	<50	108	11.2±5.5	<0.001	41	8.8±5.3	0.038	67	12.7±5.1	0.002
	50–59	133	9.3±4.9		48	7.8±4.3		85	10.1±5.0	
	60+	143	8.2±4.8		74	6.5±4.3		69	10.0±4.8	
BMI (kg/m^2)	Normal	51	8.7±5.7	0.056	31	6.2±4.7	0.09	20	12.6±5.1	0.26
	Overweight	131	8.8±5.4		79	7.3±4.8		52	11.0±5.5	
	Obese	202	10.0±4.9		53	8.5±4.4		149	10.6±4.9	
Berlin questionnaire	Normal	163	7.5±4.7	<0.001	163	7.5±4.7				
	OSA	221	10.9±5.1					221	10.9±5.1	
TSH (mU/L)	<4.5	365	9.3±5.2	0.19	156	7.3±4.7	0.1	209	10.8±5.2	0.74
	4.5 +	19	10.9±4.3		7	10.3±4.3		12	11.3±4.4	
PSS (of 56 points)	<21	177	8.2±4.7	<0.001	93	7.3±4.6	0.55	84	9.1±4.7	<0.001
	21+	203	10.4±5.3		70	7.7±4.7		133	11.8±5.1	
Sleep duration (h)	<6	121	11.0±5.5	<0.001	37	9.2±5.5	0.023	84	11.7±5.3	0.051
	6+	260	8.7±4.9		124	6.9±4.3		136	10.3±4.9	

Epworth Sleepiness Scale data are presented according to various categories listed on the left column of the table. Comparisons between groups are made using the two-sample *t* test or analysis of variance

Discussion

The salient findings of this study are that symptoms of sleepiness and fatigue experienced in association with OSA have different frequencies by gender and by race even after controlling for confounding variables such as age, BMI, thyroid function, and self-reported total sleep time. In particular, gender was the most strongly predictive variable. These findings are of obvious importance to clinicians evaluating and following subjects with OSA since patients need to be provided with the proper questionnaire tools to quantify their subjective complaints. Evaluating the symptom of fatigue with a

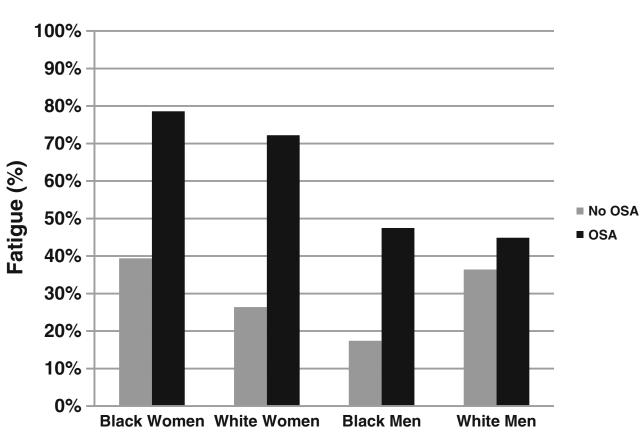

Fig. 1 Frequency of fatigue by race and gender. Fatigue associated with obstructive sleep apnea (OSA) is more commonly experienced by women than by men, $p<0.001$

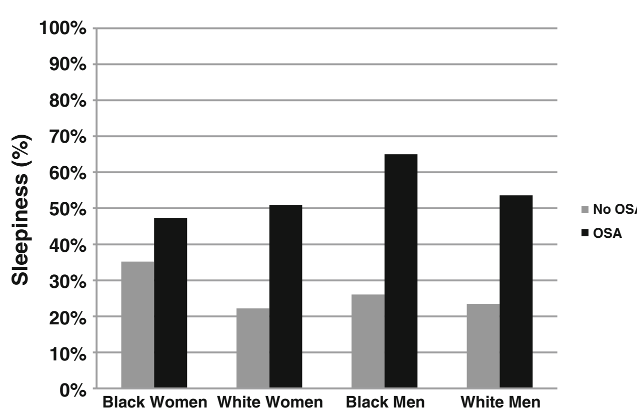

Fig. 2 Frequency of sleepiness by race and gender. Sleepiness in association with obstructive sleep apnea (OSA) is more frequently experienced by men, particularly Black men, than by all other categories, $p=0.05$

Table 4 Results of multivariate linear regression for fatigue score

Independent variables	No OSA		OSA	
	Adjusted coefficients		Adjusted coefficients	
	Beta (95 % CI)	*p* value	Beta (95 % CI)	*p* value
Age	−0.04 (−0.07 to −0.02)	<0.001	−0.03 (−0.05 to −0.004)	0.022
BMI	NS		NS	
PSS	0.09 (0.05 to 0.13)	<0.001	0.09 (0.05 to 0.12)	<0.001
Sleep duration	−0.21 (−0.45 to 0.03)	0.079	−0.14 (−0.36 to 0.09)	0.23
TSH	NS		NS	
Gender[a]	NS		−1.02 (−1.59 to 0.45)	0.001
Race[b]	NS		−0.11 (−0.72 to 0.50)	0.72

To adjust for confounding variables, multivariate linear regression was used with Fatigue Scale as the dependent variable and independent variables to include gender, race, age, BMI, PSS, TSH, and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariate models. NS indicates that a variable was not significant in univariate analysis and was therefore not included in the multivariate model

BMI body mass index, *OSA* obstructive sleep apnea, *PSS* perceived stress scale, *TSH* thyroid-stimulating hormone

[a] Females are reference group

[b] Blacks are reference group

questionnaire designed to quantify sleepiness will not suffice. Likewise, sleepiness cannot be properly evaluated with a questionnaire aimed at the symptom of fatigue. It is of major interest that a sizable proportion of the study subjects (10 to 31 % according to gender and race) experienced fatigue without sleepiness.

The proper documentation of symptoms is also important to gain appropriate allowance by insurance carriers. The National Coverage Determination for continuous positive airway pressure (CPAP) therapy published by the Centers for Medicare and Medicaid Services (CMS) sets the standard for Medicare coverage and is adopted by other insurance providers [21]. CMS considers CPAP therapy reasonable and necessary for patients with a mild category of OSA (apnea hypopnea index or respiratory disturbance index greater than or equal to five events and less than or equal to 14 events per hour) if appropriate symptoms are documented [21]. Without symptoms properly documented in these patients with a mild index of severity, their CPAP therapy would not be justifiable to insurance carriers, including CMS.

Table 5 Results of multivariate linear regression for Epworth sleepiness score

Independent variables	No OSA		OSA	
	Adjusted coefficients		Adjusted coefficients	
	Beta (95 % CI)	*p* value	Beta (95 % CI)	*p* value
Age	−0.04 (−0.09 to 0.01)	0.15	−0.03 (−0.09 to 0.03)	0.28
BMI	0.10 (−0.06 to 0.26)	0.20	NS	
PSS	NS		0.17 (0.08 to 0.25)	<0.001
Sleep duration	−0.71 (−1.27 to −0.16)	0.012	−0.19 (−0.71 to 0.33)	0.47
TSH	0.31 (−0.22 to 0.84)	0.25	NS	
Gender[a]	NS		1.59 (0.27 to 2.90)	0.018
Race[b]	−1.30 (−2.89 to 0.29)	0.11	−0.97 (−2.37 to 0.43)	0.17

To adjust for confounding variables, multivariate linear regression was used with Epworth Sleepiness Scale as the dependent variable and independent variables to include gender, race, age, BMI, PSS, TSH, and sleep duration. Separate models were examined for subjects with and without OSA. Independent variables that were significant in univariate analysis at the $p<0.25$ level were entered into the multivariate models. NS indicates that a variable was not significant in univariate analysis and was therefore not included in the multivariate model

BMI body mass index, *OSA* obstructive sleep apnea, *PSS* perceived stress scale, *TSH* thyroid-stimulating hormone

[a] Females are reference group

[b] Blacks are reference group

The finding of increased sleepiness and fatigue with shorter sleep duration conforms to prior studies that have demonstrated a strong correlation of acute and chronic sleep deprivation with decreased alertness, impaired psychomotor vigilance testing, and shorter sleep latency on mean sleep latency test [22–24]. Likewise, the observation that sleepiness and fatigue decrease with higher age groups agrees with prior research [25, 34]. We speculate that this finding of diminished symptoms with age is further explained by the circumstances that retirement and semi-retirement in older age groups allows for more opportunities to sleep and to sleep on a self-determined schedule.

The association of higher stress levels with increased symptoms of fatigue and sleepiness deserves to be addressed with further scrutiny. Potential explanations are that higher perceived stress levels intensify the experience of other symptoms such as fatigue and sleepiness. It is equally plausible that high stress levels negatively affect sleep latency, sleep continuity, and the restorative quality of sleep. These theoretical considerations warrant further study and suggest that successful stress management may be an intervention as valuable as expansion of sleep time for symptom management.

The findings of a differential experience of symptoms from disturbed sleep according to gender and race are not unique to this study. Recent reports include the observations that women more frequently experience sleep-onset insomnia than men [26] and that White women are more likely to report use of a sleep aid (prescription or nonprescription) [27]. Periodic limb movements of sleep and associated symptoms are much more common in Whites compared to Blacks [28], while estimated prevalence of narcolepsy and its symptoms are higher in women than men and in Blacks than in other racial groups [29]. Blacks are more likely to experience sleep phase advance [30] and both Blacks and women are more likely to report extremes of sleep duration (less than 5 h or greater than 9 h) [31, 32] with attendant elevation in C-reactive protein [33].

In a published review of gender differences, Ye and colleagues raise the concern that differences in symptoms on presentation with OSA may lead to the under-recognition of sleep pathology in women [15]. They note that while the Sleep Heart Health Study [34] did not find the frequency or severity of sleepiness to be affected by gender, the Wisconsin Sleep Cohort Study [1] did report a higher proportion of women with daytime sleepiness than men. Data from the Sleep Heart Health Study analyzed for impact of ethnicity but not gender [35] did find less subjective sleepiness among Blacks than Whites. Other studies report that men tend to report more sleepiness than women [36], and that women prefer to describe their subjective experience of sleep-disordered breathing using terms to denote fatigue, tiredness, and lack of energy [9, 18]. One explanation for these disparate findings regarding the different experiences of symptoms is that the questionnaire instruments may not have allowed participants, especially women, the chance to register symptoms of fatigue.

Research into the differential experience of the subjective symptoms of sleepiness versus fatigue is acknowledged to be difficult [37] and a variety of potential explanations for the disparate published reports above have been advanced. Among the explanations are that men have a less accurate perception of their pathologies than do women, that cultural influences make men less willing to acknowledge symptoms, or that there may be a gender-based neurophysiological explanation for the different experience of OSA [9]. Explanations of racial differences include the impact of socioeconomic conditions [8, 38] and varied subjective interpretation of symptoms due to differing life experiences [39]. However, there are studies that demonstrate clear anatomical differences of the upper airway according to gender and race [40]. Furthermore, a gene association study [41] and gene segregation analysis [42] have documented associations of sleep apnea vulnerability according to race.

A limitation of the current study is that subjects were categorized for the presence of sleep apnea using the Berlin Questionnaire rather than polysomnography. The Berlin Questionnaire is a reasonably sensitive and specific clinical screening tool but it is not the gold standard, suggesting that an appropriate follow-on study may be to repeat our measures in a large population with polysomnography. Another limitation is that races other than Whites and Blacks were not represented in sufficient numbers to include them in this analysis. The symptoms experienced by men and women of other races deserve further discovery.

Another factor potentially limits the ability to generalize our findings to other populations. A third of the subjects in our study sample reported fewer than 6 h of sleep per night. This degree of sleep restriction is higher than that reported in civilian populations and may be a reflection of the military culture from which our study sample derives [43]. A survey of the average sleep duration in the USA reported in 2009 that approximately 40 % of military personnel obtained less than 5 h of sleep per night compared with 8 % in the general population [43].

The data from the current study indicate that the subjective symptoms of sleepiness and fatigue are experienced not just according to gender or race but differentially by both factors simultaneously. These findings underscore the clear need to evaluate patients presenting with sleep disorders using instruments that measure more than just sleepiness and incorporate measures of fatigue and other descriptors commonly voiced by patients suffering from sleep conditions. Clinical centers evaluating patients for sleep disorders would be well advised to incorporate validated instruments for assessing symptoms of fatigue in addition to sleepiness. Future clinical guidelines should incorporate the recommendation that the evaluation of patients with sleep complaints include assessment of symptoms such as fatigue.

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Importance of Substantial Weight Loss for Altering Gene Expression During Cardiovascular Lifestyle Modification

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Objective: To examine relationships between weight loss through changes in lifestyle and peripheral blood gene expression profiles.

Methods: A prospective nonrandomized trial was conducted over 1 year in participants undergoing intensive lifestyle modification to reverse or stabilize progression of coronary artery disease. Cardiovascular risk factors, inflammatory biomarkers, and gene expression as a function of weight loss were assessed in 89 lifestyle participants and 71 retrospectively matched controls undergoing usual care.

Results: Substantial weight loss ($-15.2 \pm 3.8\%$) in lifestyle participants ($n = 33$) was associated with improvement in selected cardiovascular risk factors and significant changes in peripheral blood gene expression from pre- to post-intervention: 132 unique genes showed significant expression changes (false discovery rate corrected P-value <0.05 and fold-change ≥ 1.4). Altered molecular pathways were related to immune function and inflammatory responses involving endothelial activation. In contrast, participants losing minimal weight ($-3.1 \pm 2.5\%$, $n = 32$) showed only minor changes in cardiovascular risk factors and markers of inflammation and no changes in gene expression compared to non intervention controls after 1 year.

Conclusions: Weight loss ($\geq$10%) during lifestyle modification is associated with down-regulation of genetic pathways governing interactions between circulating immune cells and the vascular endothelium and may be required to successfully reduce CVD risk.

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Introduction

Data from the National Health and Nutrition Examination Survey indicate that 68% of adults in the United States (US) are overweight ($25 \leq \text{BMI} < 30$ kg/m^2) or obese ($\text{BMI} \geq 30$ kg/m^2) (1). Obesity is associated with significantly higher all-cause mortality in the general population (2) and is an independent risk factor for coronary artery disease (CAD) and myocardial infarction (MI) (3). If obesity continues to escalate at current rates, total healthcare costs attributable to obesity-related care could reach >$860 billion by 2030 and account for 18% of total healthcare expenditures in the US (4).

Lifestyle intervention has become an integral component of cardiovascular disease (CVD) risk reduction therapy because healthy lifestyle behaviors are effective for improving risk factors (5) and significantly reducing risk for MI (6). Weight loss in particular has been associated with positive changes in endothelial function (7)

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and markers of endothelial health (8); however, the amount of weight loss and/or the treatment regimen by which weight loss is achieved may have different metabolic effects. For example, modest weight loss of $<10\%$ over a short period of time may be sufficient to improve plasma lipid profiles and insulin sensitivity (9), but substantial long-term weight loss of $\geq 10\%$ may be necessary to significantly modulate circulating biomarkers of inflammation (10) and make clinically meaningful improvements in vascular health (11).

Clinical studies have shown that gene expression in peripheral blood is associated with coronary heart disease (12) and atherosclerotic involvement (13), but the importance of weight reduction through lifestyle modification in modulating blood-based gene expression is not well known. Our previous research showed that significant changes in the expression of genes governing processes important to vascular health occur during lifestyle modification (14), but the physiological drivers of molecular change remain unknown. In this study, we examined changes in peripheral blood gene expression as a function of weight loss during a cardiovascular lifestyle intervention to better understand molecular mechanisms by which diet and exercise affect cellular processes involved in CVD risk reduction. We hypothesized that the amount of weight loss would affect changes in traditional CVD risk factors, inflammatory molecules, and patterns of gene expression, which may influence vascular physiology and health.

Methods

Participants and intervention

A prospective, nonrandomized clinical intervention, based on the Multicenter Lifestyle Demonstration Project, was used to promote weight loss and reduction of CVD risk factors through changes in lifestyle (15). To be eligible, prospective participants were required to have CAD diagnosed by a physician or to have 2 or more risk factors. Criteria for CAD included stable angina, angioplasty, evidence of $\geq 50\%$ luminal narrowing on coronary angiogram, acute MI, bypass surgery, or stent placement; risk factors were obesity (BMI ≥ 30), hypertension (systolic pressure >140 mm Hg or diastolic pressure >90 mm Hg), high total cholesterol (>200 mg/dL), physician diagnosed diabetes, or family history of heart disease in parents or siblings. Additional acceptance criteria included physician approval and motivation to follow the program guidelines for 1 year. All patients were required to abstain from tobacco use for at least 3 months prior to enrollment and throughout the program.

Participants were required to adopt, and strictly follow for 1 year, a low fat vegetarian diet ($<10\%$ of calories from fat) with emphasis on whole grains, fruits, and vegetables, practice 1 hour of stress management per day by doing progressive relaxation, yoga, or meditation, perform 3 hours of aerobic exercise each week such as walking, cycling, rowing, or aerobics, and attend weekly group support sessions. Clinical staff met with patients twice each week during the first 3 months to orient participants to the program and maximize adherence. The remainder of the program was primarily self-directed but included ongoing weekly stress management and group support sessions.

Controls were recruited prospectively and were matched to intervention participants based on gender, age at entry within a 5-year window, and disease status (CAD or risk factors). Control subjects received standard care from their primary physicians, but did not participate in any component of the lifestyle program or receive any information, advice, or counseling regarding healthy lifestyles. This study was conducted at Windber Research Institute; the protocol and consent form were approved by the Windber Medical Center Institutional Review Board. The study is registered as NCT01805492 at ClinicalTrials.gov.

Anthropometric measurements

Data collection and reporting followed recommendations of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) group (http://www.cdc.gov/trendstatement/). Demographic and clinical information was obtained from participants and controls by standard questionnaires at the baseline and 1-year examinations. Height and weight were measured on a combined scale (Cardinal Scale, Webb City, MO, USA). Exercise capacity was determined by exercise intensity and duration during a graded treadmill exercise test.

Blood collection and plasma assays

Fasting blood samples were collected in the morning on the day of each examination and placed directly on ice. Within 2 hours of collection, whole blood was centrifuged at $\sim 1300g$ for 10 min and plasma aliquots were stored at $-80°C$. Standard lipid assays were conducted using the AEROSET™ clinical chemistry system (Abbott Laboratories, Abbott Park, IL, USA). C-reactive protein (CRP), ultra-sensitive insulin, and leptin were measured in duplicate on freshly-thawed plasma samples by radioimmunoassay (EMD Millipore, Darmstadt, Germany) at the Johns Hopkins Bayview Clinical Research Unit. Intra-assay coefficients of variation (CV%) were 6.33 for CRP, 2.69 for insulin, and 3.85 for leptin.

Dietary composition

Participants and controls completed a self-reported 72-hour dietary recall questionnaire at each examination, recording their total intake for all meals and snacks over 3 consecutive days. Reports included specific food items and drinks consumed, portion sizes, and preparation methods. Daily caloric intake and nutrient composition were then determined using Food Processor® v10.10 (ESHA Research, Salem, OR, USA).

Gene expression analysis

Peripheral blood for gene expression analysis was obtained from participants and controls at each examination using the PAXgene™ Blood RNA System (Qiagen, Valencia, CA, USA). Globin mRNA transcripts were depleted from a portion of each RNA sample using the GLOBINclear™-Human kit (Life Technologies, Carlsbad, CA, USA). Globin-depleted RNA aliquots (1 μg) were amplified using the MessageAmp™ II aRNA Amplification System (Life Technologies) and the resulting double-stranded complementary DNA was *in vitro* transcribed to synthesize amplified RNA (aRNA). Aliquots of aRNA (15 μg) labeled with biotin-11-UTP were then purified, fragmented, and hybridized to GeneChip® Human Genome U133A 2.0 arrays (Affymetrix, Santa Clara, CA, USA) and scanned on a GeneChip® Scanner 3000. RNA samples from both time points for each participant were processed together in the same batch to minimize technical artifact. The raw gene expression data have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) and are accessible through GEO Series accession numbers GSE46097 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=

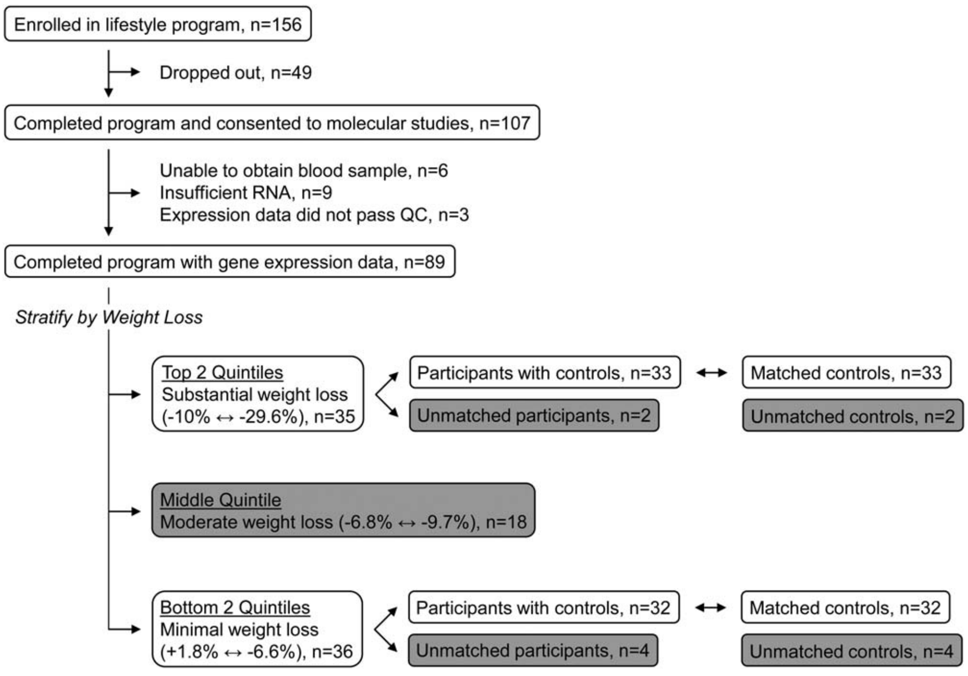

Figure 1 Flow diagram showing participant enrollment, attrition, and subgroup analysis.

GSE46097) and GSE66175 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66175).

Data analysis

Statistical analysis of traditional risk factors and biochemical variables was conducted using JMP® (v10.0). Lifestyle participants ($n=89$) were stratified into quintiles based on weight loss over 1 year (Supporting Information Table S1). The top 2 quintiles were considered "substantial weight loss" while the bottom 2 quintiles were designated "minimal weight loss." The middle quintile ($n=18$) with mean weight loss $-8.3 \pm 0.8\%$ was excluded from further analysis (Supporting Information Table S2). A flow diagram showing participant enrollment, attrition, and subgroup analysis is presented in Figure 1. For participants in the substantial ($n=33$) and minimal ($n=32$) weight loss groups with matched controls, baseline risk factor levels were then compared between participants and controls using a Wilcoxon Signed Rank nonparametric test for matched pairs. Differences in traditional risk factor response over time among the matched pairs were assessed with a matched-pairs t-test; changes in plasma biomarkers were compared between groups with a Wilcoxon Signed Rank nonparametric test.

The gene expression data were analyzed with Partek® Genomics Suite v6.5 (Partek Incorporated). Probe set intensities were obtained by robust multi-array average background correction, quantile normalization, median polish summarization, and $\log_2$ transformation. Data integrity was then assessed by standard GeneChip® quality control parameters. Duplicate blood samples indicated high data consistency; however, nine genes showing significant differences in expression among duplicate samples were excluded from further analysis (14).

Prior to longitudinal analysis, all probes ($n=22{,}215$) present on the Human Genome U133A 2.0 arrays were examined to assess levels of expression and identify potential confounding factors. Probes ($n=9{,}524$) exhibiting low levels of expression, low variance in expression, or associations with technical artifact were removed from further analyses. Using the filtered set of reliably expressed probes ($n=12{,}691$), we first compared baseline levels of gene expression between all lifestyle participants ($n=65$) and matched controls ($n=65$) in the substantial and minimal weight loss groups using two-way ANOVA. We then examined expression changes from baseline to 1 year in these weight loss groups, and separately in the respective matched controls, to determine genes that changed significantly over time in each group. Correction for multiple hypothesis testing was performed by stringent False Discovery Rate (FDR) correction following established methods (16).

Gene Set Enrichment Analysis (GSEA) was conducted in BRB-ArrayTools v4.4.0 using the BioCarta database (http://www.biocarta.com/genes/index.asp). GSEA is a functional class scoring analysis used to identify molecular pathways and transcriptional programs that are differentially expressed across networks of genes but may exhibit only subtle differences at the level of individual genes (17). This approach is more powerful for identifying differential expression compared to the more common over-representation analysis or annotation of gene lists based on individually analyzed genes. Gene sets containing more differentially expressed genes than would be expected by chance were identified using the recommended significance threshold of $P < 0.005$ (18).

Transcript validation by qRT-PCR

In participants experiencing substantial weight loss, eight genes were randomly selected for validation. Total RNA samples (200 ng) from the baseline and 1-year examinations from 27 participants with sufficient RNA remaining for analysis were subjected to qRT-PCR

using TaqMan® Gene Expression Assays (Life Technologies). Target gene expression levels were normalized to GAPDH. Duplicate samples were run for each assay and the mean value was analyzed by the $\Delta\Delta C_T$ method (19). A Pearson correlation coefficient was used to assess the relationship between fold-changes based on qRT-PCR and microarray analysis.

Results

Baseline

The average age of intervention participants (45 women and 44 men) was 60.4 years (range 40.7–85.0) and the average age of controls (36 women and 35 men) was 60.6 years (range 40.6–79.7). Despite the prospective matching strategy, participants and controls differed for some variables at baseline: lifestyle participants were heavier ($P < 0.001$), consumed a higher percentage of carbohydrates ($P = 0.034$), had lower exercise capacity ($P < 0.001$), and higher triglyceride ($P = 0.004$) and leptin ($P = 0.019$) levels (Table 1).

Weight loss and changes in risk factors

Patients experiencing substantial weight loss lost an average of 15.2 ± 3.8% of their total body weight from baseline to 1 year, while those attaining only minimal weight loss lost an average of 3.1 ± 2.5% of body weight (Table 2). The proportion of obese patients at baseline was higher ($P = 0.038$) in the substantial weight loss group (76%), but decreased to 36% by the end of the year, while remaining relatively unchanged (48% at baseline to 45% at 1 year) in the minimal weight loss group. The percentage of patients with diabetes at baseline was similar ($P = 0.775$) between the substantial (21%) and minimal (25%) weight loss groups. Patients losing substantial weight also showed significant improvement in dietary measures, diastolic blood pressure, exercise capacity, triglycerides, insulin, and leptin versus controls. Participants in the minimal weight loss group showed significant changes only for carbohydrate and fat consumption and exercise capacity, but experienced no significant changes in blood pressure, plasma lipids, or inflammatory markers compared to controls (Table 2).

Gene expression

At baseline, no genes showed a significant difference in expression between participants and matched controls using an FDR-corrected P-value of <0.05. Using the MD Anderson Cancer Center sample size calculator (http://bioinformatics.mdanderson.org/Microarray-SampleSize/), with 33 patients in the substantial weight loss group, we had 80% power to detect a ≥1.4-fold-change in gene expression. During 1 year of intensive lifestyle modification, molecular change occurred with successful weight loss—132 unique genes changed significantly in expression (FDR-corrected $P < 0.05$, fold-change ≥1.4) (Supporting Information Table S3). No expression changes were observed in participants who lost minimal weight or in nonintervention controls.

RT-PCR validation

Validation experiments showed a strong positive correlation ($r = 0.964$, $P < 0.0001$) across all eight genes between fold-changes determined by qRT-PCR and microarray analysis (Supporting Information Table S4).

TABLE 1 Dietary measures, cardiovascular risk factors, and plasma biomarkers at baseline in lifestyle modification participants and matched controls

Measure	Controls ($n = 65$)	Participants ($n = 65$)	P-value[a]
Weight (kg)	83.4 ± 15.5	95.9 ± 22.2	<0.001
BMI (kg/m^2)	28.7 ± 4.1	33.6 ± 7.6	<0.001
Dietary measures			
Calories (kcal)	1854 ± 602	2122 ± 858	0.051
% Carbohydrates[b]	50.1 ± 9.9	55.0 ± 11.9	0.034
% Fat[b]	31.2 ± 8.9	27.9 ± 10.7	0.088
Traditional risk ractors			
Systolic BP (mm Hg)	136 ± 19	137 ± 17	0.880
Diastolic BP (mm Hg)	79.9 ± 9.1	81.3 ± 10.3	0.380
Exercise capacity (Bruce)	9.8 ± 2.8	6.7 ± 2.4	<0.001
LDL cholesterol (mg/dl)	112 ± 37	112 ± 40	0.978
Total cholesterol (mg/dl)	192 ± 47	192 ± 46	0.870
Triglycerides (mg/dl)	140 ± 85	178 ± 89	0.004
Plasma biomarkers			
C-reactive protein (μg/ml)	2.9 ± 3.8	4.5 ± 5.7	0.068
Insulin (μU/ml)	15.1 ± 7.1	18.1 ± 11.2	0.195
Leptin (ng/ml)	18.5 ± 17.0	24.2 ± 20.3	0.019

BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein.
Data are mean ± SD.
[a]Based on a Wilcoxon nonparametric test.
[b]Percentage of total calories from carbohydrates or fat.

Gene set enrichment analysis

In addition to individual genes, GSEA detected 7 molecular pathways that were significantly down-regulated during successful weight loss. Many of these pathways influence interactions between circulating leukocytes and the vascular endothelium, cellular adhesion, and neutrophil granulation, which are processes important in vascular inflammation (Table 3).

Discussion

Molecular and cellular processes governing inflammation and endothelial activation are known to be important in the pathophysiology of atherosclerotic development. Given the widespread deleterious health effects of the obesity epidemic, identification of therapies that lead to sustainable weight reduction and improvement in vascular dysfunction is critical, yet few studies have examined the beneficial impact of weight loss on molecular pathways that affect endothelial function. In this study, participants in a comprehensive 1-year lifestyle modification program designed to reverse or stabilize progression of CAD showed considerable variability in weight loss, ranging from weight gain of 1.8% to loss of 29.6% of baseline body weight. Substantial weight loss led to improvement in blood pressure, triglycerides, and plasma biomarkers, as well as significant changes in peripheral blood gene expression, while minimal weight loss did not. Molecular pathways governing endothelial activation were significantly down-regulated during successful weight loss. Our observations support the hypothesis that substantial weight loss may be necessary to improve cardiovascular risk

TABLE 2 Change in dietary measures, CVD risk factors, and plasma biomarkers over 1 year in lifestyle participants and matched controls stratified by weight loss success

Measure	Weight loss group[a]	Controls Baseline	Controls One year % change	Participants Baseline	Participants One year % change	Participants vs. controls, *P*-value[b]
Weight (kg)	High	78.8 ± 14.3[d]	0.0	100.2 ± 19.6[d,e]	−15.2[f]	<0.001
	Low	87.9 ± 15.1	+1.1	91.9 ± 24.3[e]	−3.1[f]	<0.001
BMI (kg/m^2)	High	27.6 ± 3.7[d]	+0.3	34.7 ± 6.6[d]	−15.2[f]	<0.001
	Low	29.9 ± 3.9	+1.3	32.3 ± 8.7	−3.0[f]	<0.001
Dietary measures						
Calories (kcal)	High	1635 ± 548[d]	−2.6	2188 ± 850[d]	−25.3[g]	0.008
	Low	1937 ± 659	−14.3[g]	1937 ± 738	−5.7	0.324
% Carbs[c]	High	50.1 ± 9.0	−3.3	52.7 ± 10.9	+36.8[f]	<0.001
	Low	47.7 ± 10.8[d]	+5.2[g]	56.9 ± 11.6[d]	+24.0[f]	<0.001
% Fat[c]	High	31.1 ± 9.6	+5.8	30.8 ± 10.3[e]	−63.1[f]	<0.001
	Low	34.1 ± 8.8[d]	−5.8	25.8 ± 9.8[d,e]	−54.7[f]	<0.001
Traditional risk factors						
SBP (mm Hg)	High	134 ± 18	−4.3[g]	135 ± 16	−7.3[g]	0.351
	Low	138 ± 19	−8.3[f]	139 ± 17	−7.4[g]	0.775
DBP (mm Hg)	High	77.9 ± 9.2	−1.1	81.2 ± 11.1	−10.2[f]	0.008
	Low	81.7 ± 8.6	−5.1[g]	81.8 ± 9.5	−7.2[g]	0.374
EC (Bruce)	High	9.9 ± 3.0[d]	+0.7	6.8 ± 2.0[d]	+44.0[f]	<0.001
	Low	9.7 ± 2.8[d]	−0.9	6.8 ± 2.4[d]	+28.3[f]	<0.001
LDL (mg/dl)	High	109 ± 38	−2.1	112 ± 40	−0.2	0.792
	Low	115 ± 37	−1.0	112 ± 40	−1.2	0.972
TCH (mg/dl)	High	191 ± 52	−0.7	193 ± 43	−4.3	0.447
	Low	193 ± 42	+0.4	191 ± 50	−2.6	0.469
TG (mg/dl)	High	144 ± 108[d]	+9.8	190 ± 107[d]	−16.9[g]	0.022
	Low	135 ± 52[d]	+11.7	166 ± 65[d]	+3.2	0.602
Plasma biomarkers						
CRP (μg/ml)	High	2.2 ± 1.5[d]	−2.0	4.1 ± 3.5[d]	−32.1[f]	0.071
	Low	3.6 ± 5.1	−15.1	4.8 ± 7.3	−31.5	0.269
Insulin (μU/ml)	High	13.8 ± 6.3[d]	+3.4	21.5 ± 12.4[d,e]	−35.1[f]	<0.001
	Low	16.9 ± 7.9	+4.7	15.0 ± 9.3[e]	−1.5	0.540
Leptin (ng/ml)	High	16.9 ± 12.1[d]	+5.4	24.5 ± 15.2[d]	−50.9[f]	<0.001
	Low	20.3 ± 20.9	+12.1	24.0 ± 24.8	−10.4	0.101

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; EC, exercise capacity; LDL, low-density lipoprotein; TCH, total cholesterol; TG, triglycerides; CRP, C-reactive protein.
Data are mean ± SD.
[a]High, substantial weight loss ($n = 33$); Low, minimal weight loss ($n = 32$).
[b]Based on a matched-pairs t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers) comparing changes from baseline to 1 year in participants versus matched controls.
[c]% Carbs is percentage of total calories from carbohydrates; % Fat is percentage of total calories from fat.
[d]Participants and controls significantly different at baseline ($P<0.05$) based on a Wilcoxon Signed Rank test for matched pairs.
[e]Substantial weight loss and minimal weight loss participants significantly different at baseline ($P<0.05$) based on a Wilcoxon nonparametric test.
[f]$P<0.001$ compared to baseline using a paired t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers).
[g]$P<0.05$ compared to baseline using a paired t-test (dietary and traditional risk factors) or Wilcoxon Signed Rank test (plasma biomarkers).

beyond what traditional biomarkers reveal. Improvement in vascular health may require molecular attenuation of interactions between circulating immune cells and the vascular endothelium, which can potentially be achieved with substantial weight loss.

Heightened oxidative stress and elevated levels of circulating inflammatory cytokines are associated with metabolic abnormalities including insulin resistance and diabetes (20). In obese patients, vascular inflammation, impaired endothelial function, and reduced arterial responsiveness (21,22) lead to accelerated rates of atherosclerosis and a higher incidence of major cardiovascular events compared to healthy-weight individuals (23). Although weight loss through diet and/or exercise appears to be the most appropriate therapy to reverse vascular abnormalities associated with obesity (24,25), weight loss

TABLE 3 Molecular pathways differentially expressed over 1 year in lifestyle participants experiencing substantial weight loss

Pathway ID	Pathway name	No. genes	Function	Direction	*P*
h_ahsp	Hemoglobin's chaperone	10	Hemoglobin biosynthesis and stability	Down	0.0002
h_monocyte	Monocyte and its surface molecules	9	Immune/inflammatory response; monocyte interaction with vascular endothelium	Down	0.0014
h_neutrophil	Neutrophil and its surface molecules	7	Immune/inflammatory response; neutrophil interaction with vascular endothelium	Down	0.0021
h_lymphocyte	Adhesion molecules on lymphocyte	7	Immune/inflammatory response; lymphocyte interaction with vascular endothelium	Down	0.0023
h_bArrestin-src	Roles of β-arrestin-dependent recruitment of Src kinases in GPCR signaling	12	Endocytosis; cell proliferation; neutrophil degranulation	Down	0.0023
h_granulocytes	Adhesion and diapedesis of granulocytes	14	Immune/inflammatory response; granulocyte interaction with vascular endothelium	Down	0.0037
h_integrin	Integrin signaling pathway	23	Intracellular signaling; cellular adhesion, mobility, and progression through cell cycle	Down	0.0044

The LS permutation *P*-value for all pathways was < 0.005. GPCR, G protein coupled receptor. Pathways from the BioCarta database available at http://www.biocarta.com/genes/index.asp.

has not been consistently associated with improvements in endothelial function (26), possibly due to differences in the percentage of weight loss achieved by patients across studies. Recent data suggest that the amount of weight reduction may be critical to achieving and maintaining healthy vascular function (27).

Changes in plasma insulin and leptin levels during lifestyle modification corroborated the hypothesis that substantial weight loss may be necessary to produce beneficial anti-inflammatory and anti-oxidative effects on the vasculature. Insulin has been shown to stimulate expression of adhesion molecules (28) and leptin is known to

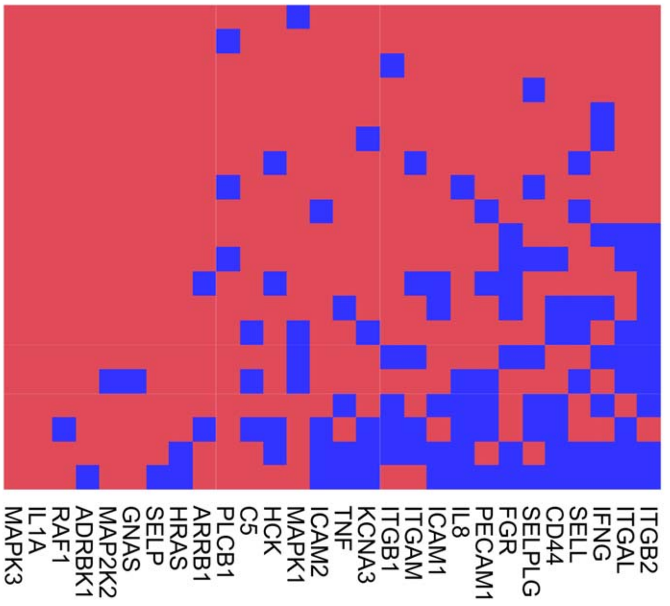

Figure 2 Blue squares denote genes comprising immune and inflammatory pathways, which were significantly down-regulated during substantial weight loss in this study, that are expressed in various subpopulations of human leukocytes. References are provided in Supporting Information Table S6.

promote proliferation and activation of T-lymphocytes, which may contribute to endothelial dysfunction in overweight and obese patients by inducing oxidative stress on endothelial cells (29). In this study, lifestyle participants who lost substantial weight ($\sim$15% on average) showed significant reductions in insulin and leptin levels compared to matched controls, but participants losing minimal weight (3% on average) showed no significant changes in circulating markers of inflammation.

Previous research has documented expression changes in genes influencing immune response and vascular inflammation in peripheral blood (30-32) and adipose tissue (33,34) following diet and exercise. Similarly, the genomic response to substantial weight loss during lifestyle change in this study involved down-regulation of genes and pathways associated with endothelial function. Polarization of lymphocytes toward an atherogenic phenotype has been observed in obese patients (35) and changes in the relative abundance of circulating immune cells have been shown to occur in response to long-term weight loss (36). These observations suggest that participants in this study showing substantial weight loss may have experienced shifts in certain leukocyte populations, which may have contributed to changes in peripheral blood gene expression.

Peripheral blood is a complex tissue with diverse cell populations whose relative abundance is dynamic over time. Although whole blood RNA isolation systems such as PAXgene cannot distinguish expression patterns unique to specific subpopulations of circulating cells, they are designed, and have successfully been used, to accurately capture *in vivo* transcription profiles. To address the specificity of our blood-based gene signature approach, we examined expression profiles reported in the literature for major leukocyte subpopulations as well as several control tissues (human brain, liver, pancreas) (37). Only 4 of the 132 genes showing differential expression in peripheral blood during substantial weight loss were elevated in the control tissues, suggesting enrichment for specific subsets of leukocytes during this study. Many genes comprising the differentially expressed BioCarta pathways related to immune and inflammatory responses were expressed in a variety of leukocyte types, but mainly in activated T-cells and other T-cell populations (Figure 2). These specialized cells exhibit different patterns of gene expression that govern their participation in various types of immune responses (38). Activation of T-cells in particular has a major influence on gene expression and is usually associated with production of cytokines and adhesion molecules, which is an important early step in the development of atherosclerosis. Endothelial activation, characterized by adhesion of circulating leukocytes to the vascular endothelium and transmigration across the endothelial barrier, also produces significant changes in gene expression (39). Our data thus suggest that one mechanism through which substantial weight loss may affect vascular health is the down-regulation of molecular pathways associated with endothelial activation and vascular inflammation. Dysregulation of these inflammatory pathways may be attributable to altered transcription within certain leukocyte subpopulations and/or changes in the relative abundance of specialized immune cell types during weight loss.

The prospective, longitudinal nature of this study with validated protocols and matched controls minimized sources of bias and confounding and improved our ability to assess the effects of weight loss on molecular processes relevant to vascular health. The plasma biomarker data strengthened the conclusion that weight loss was an important driver of molecular change. Due to demanding behavioral changes and significant time commitment necessary to successfully complete the intervention, it was impractical to use a randomized study design. We analyzed the data using a per-protocol approach, but included all patients who completed the program whether or not they strictly adhered to the program guidelines. There were no significant differences in the average compliance scores or the percentage of participants meeting compliance targets at the 1-year examination for diet, exercise, and stress management between participants who lost substantial weight and those who lost minimal weight (Supporting Information Table S5); however, adherence data were self-reported by the participants and thus subject to inherent inaccuracies. Because the lifestyle intervention focused on a combination of dietary modification, exercise, and stress reduction, we could not quantify the relative contribution of each modality to overall weight loss and molecular change.

Conclusion

To our knowledge, this is the first study to demonstrate that substantial weight loss during lifestyle modification for improved cardiovascular health is associated with changes in peripheral blood gene expression. Conversely, there were no significant molecular changes associated with minimal weight loss. Weight reduction of at least 10% was associated with significant down-regulation of genetic pathways governing interactions between circulating immune cells and the vascular endothelium. The observed changes in gene expression with substantial weight loss may improve endothelial function and produce meaningful vascular health benefits. As peripheral blood gene expression profiles reflect the pathophysiology of the vasculature, an increased understanding of leukocyte gene expression is necessary to identify mechanisms through which weight loss affects cellular processes involved in cardiovascular risk reduction. Further studies are needed to quantify the effects of weight loss on endothelial function and vascular health. O

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A Systematic Approach Incorporating Family History Improves Identification of Cardiovascular Disease Risk

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Background: Although family history (FH) is an independent predictor of cardiovascular disease (CVD) risk, traditional risk scores do not incorporate FH. Nurse practitioners routinely solicit FH but have no mechanism to incorporate the information into risk estimation. Underestimation of risk leaves clinicians misinformed and patients vulnerable to the CVD epidemic. **Objective:** We examined a systematic approach incorporating FH in CVD risk assessment, validating risk reclassification using carotid intima-media thickness (CIMT), a surrogate measure of atherosclerosis. **Methods:** Of 413 consecutive patients prospectively enrolled in the Integrative Cardiac Health Project Registry, a subgroup of 239 was low or intermediate risk by the Framingham Risk Score. A systematic approach for the assessment of FH was applied to this subgroup of the registry. A positive FH for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, conferred reclassification to high risk. Reclassification was validated with CIMT results. **Results:** Chart audits revealed adherence to the systematic approach for FH assessment in 100% of cases. This systematic approach identified 115 of 239 (48%) patients as high risk because of positive FH. Of the reclassified patients, 75% had evidence of subclinical atherosclerosis by CIMT versus 55% in the patients not reclassified, $P < 0.001$. Logistic regression identified positive FH for premature CVD (odds ratio, 2.6; $P = 0.001$) among all variables, as the most significant predictor of abnormal CIMT, thus increasing risk for CVD. **Conclusions:** The Integrative Cardiac Health Project systematic approach incorporating FH into risk stratification enhances CVD risk assessment by identifying previously unrecognized high-risk patients, reduces variability in practice, and appropriately targets more stringent therapeutic goals for prevention.

KEY WORDS: cardiovascular disease, family history, primary prevention, risk assessment

Cardiovascular disease (CVD) is the leading cause of death and disability in the United States and Europe.[1,2] On the basis of numerous analyses performed to determine the thresholds for increased risk, family history (FH) of premature CVD is defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women.[3–12] With this definition, FH of premature CVD is an independent and robust predictor of risk. When FH is positive, individual risk for CVD is increased by as much as 5-fold.[10] Although US and European guidelines include positive FH as a high-risk factor, traditional risk scoring systems do not. Nurse practitioners routinely inquire about FH in clinical practice, but there is variability in the approach to capture and interpret the data.[5,13,14]

The Framingham Risk Score (FRS), the most widely used CVD risk assessment tool, significantly underestimates risk because it does not incorporate FH data.[15,16] Studies show FRS to be only 50% accurate in identifying patients at high risk for heart disease.[15] In fact, up to 75% of patients experiencing an acute coronary syndrome are assessed as low risk by the FRS.[17] When FH is not used in risk assessment, a large subgroup of the population at risk for CVD remains unrecognized, leaving them unaware of their threatened health status. Failing

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to identify these high-risk individuals precludes clinicians from prescribing targeted and risk-specific self-care interventions aimed at CVD prevention.[13]

Although FH has been repeatedly demonstrated to be a high risk factor of CVD, current guidelines provide no mechanism for the systematic collection, interpretation, and risk score adjustment using this information. We implemented a systematic approach for the assessment of FH to standardize identification of high-risk patients and used carotid intima-media thickness (CIMT) to validate the high-risk reclassification.[18,19]

Methods

This investigation was conducted with the approval of the institutional review board at Walter Reed National Military Medical Center in Bethesda, Maryland. The study design is a subgroup analysis of data prospectively collected on consecutive patients enrolled in the Integrative Cardiac Health Project (ICHP) Registry. The ICHP Registry is a CVD prevention program operating in a research Center of Excellence for the US Department of Defense. All subjects gave informed consent for participation in the registry, and the study was conducted according to the principles stated in the Declaration of Helsinki.

The ICHP offers military healthcare beneficiaries a 6-month tailored CVD risk reduction program. Patients who join the program by self or provider referral must be adults older than 17 years. All patients seen at the ICHP are categorized upon baseline assessment as low, intermediate, or high risk for CVD by the FRS. In addition, ICHP patients receive results of a detailed CVD risk assessment and a personalized preventive health plan. As part of the ICHP Registry, patients receive a CIMT, which is maintained as a long-term CVD outcome measure. The CIMT findings are not used to calculate the patient's CVD risk status. The following variables were collected on all patients who attended the ICHP from 2008 to 2011: age, gender, ethnicity, FRS, FH status, CIMT and diagnoses of CVD, hypertension, dyslipidemia, and diabetes.

Upon entry to the ICHP, patients undergo a cardiovascular-focused history and physical examination. Medical history, including smoking history, is elicited with a written question as part of a questionnaire, and the responses are verified verbally by a nurse practitioner at the time of the physical examination. medical history such as hypertension, diabetes, and dyslipidemia is also elicited on the questionnaire, validated verbally by a nurse practitioner and reconciled with data recorded in the patient's medical record. Body mass index (BMI) is calculated with the formula kilograms divided by the square of height in meters using measured height and weight from a medical-grade weight scale and stadiometer. Blood pressure is first measured after the patient has been sitting quietly for 5 minutes using a GE DINAMAP PRO Series 100–400V2. Five minutes later, a second blood pressure reading is taken, and the 2 values are averaged for the record. All cardiovascular-relevant laboratory data are obtained in the blood chemistry laboratory at the medical facility, with the laboratory certified by the Clinical Laboratory Improvement Amendments.

At a subsequent appointment, the patients were informed of their CVD risk status and were provided therapeutic goals specific to their determined risk category. Although the patients in all risk categories (low, intermediate, and high) received recommendations for healthy behavior change, the high-risk patients were targeted with aggressive treatment goals for cholesterol, blood pressure, and weight management.

This analysis was limited to a subgroup of ICHP patients whose calculated FRS showed low or intermediate 10-year risk because the high-risk patients could not be reclassified to a higher level of risk. Diabetes is considered by the FRS to be a high-risk factor, and therefore, any patient with diabetes was excluded from this analysis.

Risk Assessment (Carotid Intima-Media Thickness)

The CIMT findings were reviewed and evaluated by 1 sonographer oriented to the purposes of the project but blinded to the FH information for each patient. Images were obtained on a single ultrasound machine (SonoSite MicroMaxx 3.4.3; Bothell, Washington) using a linear array 5- to 10-MHz transducer with standardized image settings, including resolution mode, depth of field, gain, and transmit focus. All sonograms were obtained with the patients supine with the head facing the contralateral side. Electrocardiograms were recorded simultaneously. The sonographer, also trained in the measurement of CIMT, performed the analyses with commercially available software (Sonocalc IMT, Bothell, Washington). Carotid intima-media thickness was determined from images of the far wall of the distal common carotid arteries (immediately proximal to the carotid bulb) and reported as the mean value for the bilateral measurement. The near (intimal-luminal interface) and far (medial-adventitial interface) field arterial wall borders were manually traced for measurement of mean CIMT (millimeters) across a 10-mm arterial segment. A mean CIMT measurement of greater than the 75th percentile cutoff value, based on age and gender, in at least 1 carotid vessel was defined as an abnormal CIMT, as proposed by the American Society of Echocardiography Carotid Intima-Media Thickness Task Force.[20] This cutoff value has been used in a prior large atherosclerosis outcomes study, the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) Study, with CIMT as its main outcome measure.[21]

Impact Assessment

For CVD risk assessment, ICHP nurse practitioners evaluated FRS and FH status. The FRS, which takes into account age, gender, smoking, systolic blood pressure, total cholesterol, and high-density cholesterol levels, was determined using a web-based tool.[22] A systematic approach to evaluating FH was applied to standardize risk stratification beyond the FRS (see Figure). The ICHP nurse practitioners were trained using a standardized operating procedure (SOP) detailing the collection of FH during the initial assessment of each patient. This SOP defined positive FH of premature CVD as a first-degree relative (parent or sibling) having a CVD event before the age of 55 years in men and 65 years in women.[11,12] Cardiovascular disease events included myocardial infarction; cardiovascular revascularization; and diagnosis of coronary disease, stroke, or transient ischemic attack. The family tree was explored in detail for these CVD events, specifically in first-degree relatives and for the age of occurrence. Any first-degree family member meeting these criteria conferred a high-risk designation irrespective of the FRS result. Patients who were unable to provide FH (for example, patients who are adopted and do not have FH information) were excluded from the analysis. Chart audits were performed on 100% of cases to verify adherence to the systematic approach outlined in the SOP.

Analyses were performed using the Statistical Package for the Social Sciences (version 20.0).[23] Descriptive and frequency statistics were presented as mean (SD) or percentage. Student *t* test for continuous variables and χ^2 analysis for categorical variables were used. Logistic regression was performed to assess the predictive impact of factors on the likelihood of a patient having an abnormal CIMT.

Results

Of 413 patients, 19 patients (4.6%) were excluded for lack of FH data, leaving 394 for this analysis. Using the FRS, 239 of 394 patients (61%) were classified as low or intermediate risk. Frequency and descriptive analyses revealed a normally distributed population by age with no missing data. Demographic findings showed a mean age of 49 years (range, 20–76); 59% were women; 51%, white; 25%, black; 6%, Hispanic; and 1%, Asian, with 17% undeclared or other. The mean body mass index was 30.5 kg/m^2. The population was characterized by hypertension (40%), dyslipidemia (71%), and smoking (2%).

Chart audits revealed adherence to the systematic approach for FH assessment in 100% of the 239 patients who were in the low or intermediate FRS category. The systematic approach identified 115 of 239 patients (48%) as having positive FH for CVD. Table 1 displays the comparison between the 2 groups (positive FH and negative

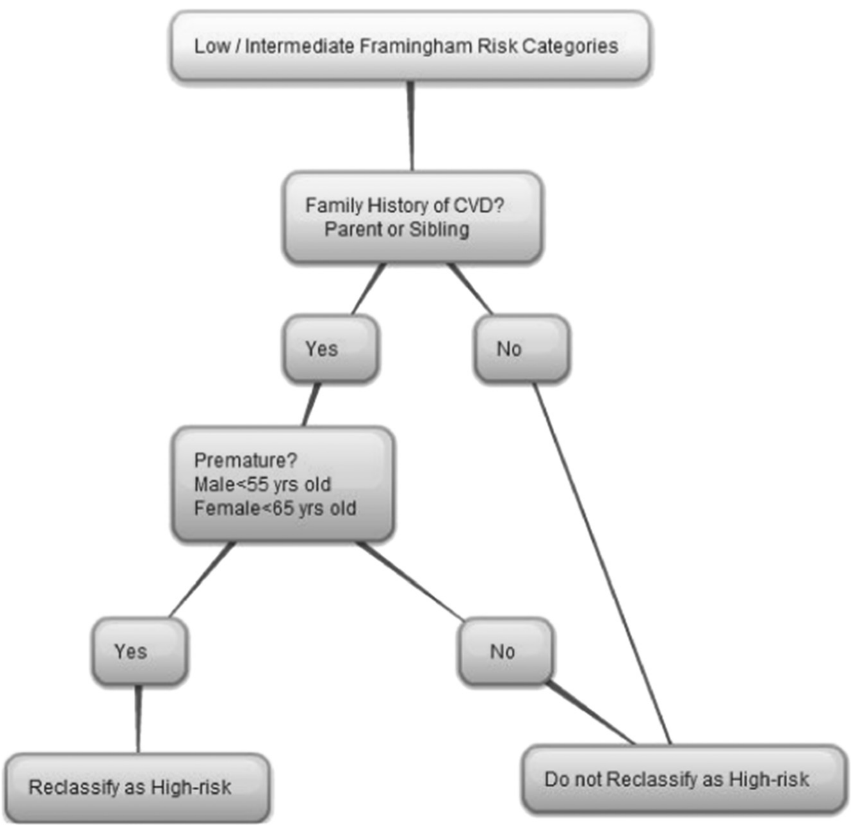

FIGURE. *The Integrative Cardiac Health Project systematic approach incorporating family history in CVD assessment.*

TABLE 1 Baseline Characteristics of Population at Low and Intermediate Cardiovascular Disease Risk

N = 239	Negative FH, n = 125	Positive FH, n = 114	*P*
Age, y	44.9 (12.18)	54.3 (10.16)	0.02[a]
Gender (female)	55%	64%	0.17
BMI, kg/m^2	29.5	31.3	0.39
Active smoker	3%	2%	0.86
Hypertension	31%	39%	0.18
Dyslipidemia	74%	70%	0.47
FRS	3.01 (3.21)	4.5 (4.19)	0.001[a]
Glucose, mg/dL	89.8 (10.1)	92.8 (9.68)	0.84
CIMT (abnormal)	55%	75%	<0.001[a]

Data are presented as mean (SD) or percentage. *t* test is used for continuous variables. χ^2 analysis is used for categorical variables. *P* values are given for the comparison between FH groups.
[a]Denotes statistical significance.

FH). Between FH groups, age, FRS, and CIMT were different. The patients with a positive FH were older (54.3 vs 44.9 years, $P = 0.02$). The mean FRS scores were statistically different (positive FH, 4.5; negative FH, 3.0; $P < 0.001$), although this difference is not clinically important because both scores indicate low risk. In validating the reclassification using CIMT, the proportion of patients with an abnormal CIMT was clinically and statistically different between groups, with a higher percentage in the positive FH group (75% vs 55%, $P < 0.001$). No effect of confounding was detected because there was no difference between groups using χ^2 analysis for gender, BMI, smoking history, hypertension, and dyslipidemia.

Logistic regression was performed to assess the impact of factors on the likelihood that patients would have an abnormal CIMT (Table 2). The model contained 5 independent variables (race, gender, FH category, diagnoses of hypertension and dyslipidemia). Age was not included in the model because age is one of the normative factors used as a cutoff value in the definition of normal versus abnormal CIMT.[20] The full model containing all predictors was statistically significant, χ^2 (11, n = 239) = 41.1, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT. The model as a whole explains between 16% and 22% of the variance in CIMT status and correctly classified 69% of cases after inclusion of the predictors. Two of the independent variables made a unique statistically significant contribution to the model (black race: odds ratio [OR], 5.8; $P = 0.02$; 95% confidence interval [CI], 1.3–26.9, and presence of positive FH: OR, 2.4; $P = 0.006$; 95% CI, 1.3–4.5). In an effort to find the most parsimonious model predicting abnormal CIMT,[24] logistic regression was repeated using the 2 contributing variables, black race and presence of positive FH. This new model containing the 2 predictors was statistically significant, χ^2 (6, n = 239) = 28.6, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT. The model as a whole explains between 11% and 16% of the variance in CIMT status and correctly classified 69% of cases after inclusion of the predictors. Although black race was no longer a significant predictor in the new model, presence of positive FH remained the only significant predictor contributing to the logistic regression model (black race: OR, 0.528; $P = 0.290$; 95% CI, 0.162–1.725, and presence of positive FH: OR, 2.64; $P = 0.001$; 95% CI, 1.47–4.73). The Hosmer-Lemeshow test showed goodness of fit with a significance of 0.86.

Discussion

Although national guidelines recognize the importance of FH for CVD risk, these guidelines provide no mechanism to instruct practitioners on how to translate this FH information to a more accurate determination of risk for the individual patient.[1,2,5] In fact, there has been

TABLE 2 Logistic Regression Model

							95% CI for OR	
Predictors of Abnormal CIMT	*B*	SE	Wald	*df*	*P*	OR	Lower	Upper
Black race	1.761	0.781	5.088	1	0.024[a]	5.816	1.260	26.856
Gender	0.441	0.318	1.921	1	0.166	1.554	0.833	2.897
FH positive	0.883	0.318	7.691	1	0.006[a]	2.418	1.296	4.513
Diagnosis of hypertension	0.540	0.346	2.435	1	0.119	1.716	0.871	3.382
Diagnosis of dyslipidemia	0.196	0.347	0.320	1	0.572	1.217	0.616	2.404
Constant	−1.736	0.808	4.612	1	0.032	0.176		

The model contained 5 independent variables (race, gender, positive FH, diagnosis of hypertension, and diagnosis of dyslipidemia). The full model containing all predictors was statistically significant, χ^2 (11, n = 239) = 41.1, $P < 0.001$, indicating that the model was able to distinguish between normal and abnormal CIMT.
[a]Denotes statistical significance.

a call for evidence on the value of systematically using FH in CVD risk assessment.[5]

Investigation of FH requires a systematic approach in which there is minimized variability in assessment of risk among clinicians because there are numerous criteria needed to fulfill the definition of positive FH. These criteria are complex and require an in-depth review of the family tree including gender, relationship to the patient, and age of onset of CVD. A simple yes/no question is inadequate to provide the relevant data to illicit an accurate FH for risk estimation.[5]

Our study population of mostly overweight, late–middle-aged subjects with a variety of races is fairly typical of a population seeking medical evaluation for CVD risk estimation. One risk factor that makes our sample population stand out as different from the US population is the very low prevalence of self-reported smoking behavior (2%), which is substantially lower than US norms (19%).[25] A potential explanation for this discrepancy is that there have been initiatives for health promotion that champion smoking cessation, including a ban of smoking on site in the medical facility. Furthermore, self-referred patients seeking wellness in a CVD risk reduction program may also be less likely to smoke.

We have shown that, among asymptomatic, previously low- or intermediate-risk patients by FRS, the use of a systematic approach for the incorporation of FH resulted in identifying a substantial proportion of patients at high risk for CVD. These patients would have otherwise been told that they were not at high risk for CVD. In addition, we have demonstrated the feasibility of implementing a systematic approach for incorporating FH, an easily accessible and inexpensive data point.[26]

The validity of this reclassification was substantiated using CIMT in the positive FH group to find 75% abnormal CIMT results compared with 55% abnormality in the group with negative FH. This is consistent with findings from the Framingham Offspring Study, a large population-based cohort of families in which CVD events were validated prospectively in both parents and offspring.[11] On the basis of that study, an association was found between parental history and subclinical atherosclerosis among offspring measured by CIMT.

Our study highlights the predictive value of including FH in assessment of risk for CVD. By logistic regression, positive FH was shown to be a robust predictor, indicating that patients with presence of positive FH were more than twice as likely to have an abnormal CIMT compared with those with negative FH, when controlling for all other factors in our data set. Although positive FH was an independent predictor, other factors including age, race, gender, and diagnoses of hypertension and dyslipidemia were not predictors of an abnormal CIMT. This may be explained by an underlying atherosclerotic mechanism causing functional abnormalities in offspring of patients with premature CVD, independent of known vascular risk factors.[27–29]

The mean age of the patients with a positive FH was greater than of the patients with negative FH in our cohort. This finding may be explained by the fact that older study subjects will have older siblings who are more likely to have experienced a cardiovascular event and younger study subjects will more likely have younger siblings who have not yet developed CVD. The older sibling's event gives the older study subject a positive FH, whereas younger study subjects are more likely to have a negative FH.

The lack of a mechanism to incorporate FH in CVD risk assessment is a major gap in current practice. This article suggests a systematic approach to translate the evidence for FH into clinical practice. When patients at high risk for CVD are properly identified, they are given appropriate therapeutic goals to match their heightened risk category, and more attention is paid to healthy lifestyle behavior change. Ultimately, incorporating FH in risk assessment is a way to personalize preventive therapies aimed at combating the epidemic of CVD.

Limitations

Limitations include the use of CIMT as a surrogate measure for CVD events. However, this is a commonly used strategy to overcome expense, feasibility issues, and risk associated with radiological studies such as electron beam computerized tomography and computed tomographic angiography.[18]

Although our sample population shows some characteristics that mirror the US population generally such as overweight,[30] an important characteristic that deviates from the US population is the very low prevalence of smoking status (2%). This difference may limit our ability to generalize our findings to the population at large. Another potential limitation may be referral bias because patients with positive FH may have a heightened sense of concern regarding their CVD health before entering the program.

Furthermore, data collection did not include all individual variables thought to influence CVD, although variables necessary for FRS calculation were captured. A further limitation is that approximately 5% of our patients were unable to provide FH.

Conclusions

Translation of evidence into practice is dynamic, and mechanisms to help clinicians accomplish translation continue to evolve. Recent evidence indicates that positive FH has predictive validity.[4] This study demonstrates that a reproducible systematic approach for adding FH to current practice enhances predictive value and identifies high-risk patients who, at present, are not captured.

This report describes a mechanism that addresses a current gap in clinical practice. The findings of this report are sufficiently promising to warrant further implementation and validation in other settings, using different study designs and outcome measures.

What's New and Important

- Family history for premature CVD, defined as a first-degree relative having a CVD event before the age of 55 years in men and 65 years in women, confers a high-risk classification for CVD as validated by a surrogate marker of atherosclerosis.
- A systematic approach for incorporation of FH for premature CVD will enhance the identification of high-risk patients.
- Incorporating FH in risk assessment is a way to personalize preventive therapies aimed at combating the epidemic of CVD.

We urge practitioners to adopt a systematic approach to incorporate FH in CVD risk assessment to provide patients with more accurate risk stratification and to target preventive interventions for high-risk individuals. We believe that implementation of such a systematic approach would have a global impact on patients at risk for CVD.

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Early Empowerment Strategies Boost Self-Efficacy to Improve Cardiovascular Health Behaviors

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Abstract

Background: Self-efficacy, defined as confidence in the ability to carry out behavior to achieve a desired goal, is considered to be a prerequisite for behavior change. Self-efficacy correlates with cardiovascular health although optimal timing to incorporate self-efficacy strategies is not well established. We sought to study the effect of an empowerment approach implemented in the introductory phase of a multicomponent lifestyle intervention on cardiovascular health outcomes.

Design: Prospective intervention cohort study

Methods: Patients in the Integrative Cardiac Health Project Registry, a prospective lifestyle change program for the prevention of cardiovascular disease were analyzed for behavioral changes by survey, at baseline and one year, in the domains of nutrition, exercise, stress management and sleep. Self-efficacy questionnaires were administered at baseline and after the empowerment intervention, at 8 weeks.

Results: Of 119 consecutive registry completers, 60 comprised a high self-efficacy group (scoring at or above the median of 36 points) and 59 the low self-efficacy group (scoring below median). Self-efficacy scores increased irrespective of baseline self-efficacy but the largest gains in self-efficacy occurred in patients who ranked in the lower half for self-efficacy at baseline. This lower self-efficacy group demonstrated behavioral gains that erased differences between the high and low self-efficacy groups.

Conclusions: A boost to self-efficacy early in a lifestyle intervention program produces significant improvements in behavioral outcomes. Employing empowerment in an early phase may be a critical strategy to improve self-efficacy and lower risk in individuals vulnerable to cardiovascular disease.

Keywords: cardiovascular diseases; health behavior; lifestyle; prevention; risk factors; risk reduction; self-efficacy

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in Westernized nations (World Health Organization, 2010). Patients living with CVD experience decreased quality of life (Lewis et al., 2014), increase their utilization of health care resources (Tung et al., 1999) and decrease their economic productivity (Meland, Grønhaug, Oystese, & Mildestvedt, 2011). CVD prevention has therefore become a major goal of health care systems and medical professional societies (Eckel et al., 2014).

The main strategy in CVD prevention is to identify and improve risk factors (Eckel et al., 2014). Sustained improvements in CVD risk reduction requires that patients be made aware of their individual risk factors as well as their lifestyle behaviors that affect those risk factors such as avoiding tobacco use, making healthful nutrition choices, getting adequate exercise, managing stress levels, and getting adequate quantity and quality of sleep.

However, awareness alone is not adequate to change lifestyle behaviors affecting CVD risk (Elis et al., 2008), (Scotto, Waechter, & Rosneck, 2011). For behavior change, social-cognitive theory has proposed the concept of self-efficacy, defined as a person's belief in his/her ability to carry out behavior to achieve a desired goal (Bandura, 1977). A number of studies have shown that self-efficacy is a prerequisite for making behavioral changes for the self-management of chronic conditions such as hypertension (Criswell, Weber, Xu, & Carter,

2010), (Warren-Findlow, Seymour, & Brunner Huber, 2012), overweight (Linde, Rothman, Baldwin, Jeffery, 2006), (Roach, et al., 2003), and addictions (Kadden & Litt, 2011).

Integral to a collaborative care model for chronic disease is patient empowerment, which is defined as helping patients to develop the inherent capacity to be responsible for one's own life (Funnell & Anderson, 2003). Empowerment approaches include interactive teaching strategies designed to involve patients in problem solving and as a result impact self-efficacy. Although studies support the utility of this approach, health professionals need a way to operationalize the empowerment of patients (Anderson & Funnell, 2005). To lower CVD risk and improve adherence to healthy lifestyle change, strategies must be implemented to empower patients by enhancing self-efficacy.

There is an inverse relationship between self-efficacy and CVD risk factor profiles (Bailey, Kashani, Eliasson, & Vernalis, 2013), (Eliasson et al., 2015). However, the importance of self-efficacy for the management of CVD risks is not well established. Prior studies on this patient have shown mixed results. An observational study showed strong associations of high self-efficacy and adherence to two of four healthful behaviors for CVD (Sol, van der Graaf, van Peterson, & Visseren, 2011). A sub-analysis of a large prospective trial for treatment of hypertension showed that self-efficacy scores could not predict behavior change (Wingo et al., 2013). One randomized trial showed a lack of power for self-efficacy to predict adherence to 6 of 9 healthful behaviors (Sol, van der Graaf, van der Bijl, Goessens, & Visseren, 2008) and a second randomized trial showed equivocal results of an intervention to increase self-efficacy for exercise in cardiac rehabilitation patients (Barkley & Fahrenwald, 2013). Little is known about the appropriate timing or mechanism for the implementation of self-efficacy enhancing strategies to achieve successful behavior change.

In the present study, we investigated the impact of an intervention designed to enhance self-efficacy by giving patients an early boost using an empowerment approach to improve adherence to healthy lifestyle behaviors. The empowerment intervention was implemented as part of a cardiovascular (CV) health program targeting behaviors in the areas of nutrition, exercise, perceived stress and sleep.

2. Methods

The Integrative Cardiac Health Project (ICHP) is a prospective registry of patients enrolling in a 12-month CV health program. The study has been registered with clinicaltrials.gov and may be found using identifier NCT01975181. All patients give informed consent for participation in the registry and the study is being conducted according to the principles stated in the Declaration of Helsinki.

Patients are self-referred or referred by a healthcare provider to assess their CVD risks and to learn how those risks can be improved through lifestyle behavior changes. Patients participating in ICHP are men and women over 17 years of age who are eligible for care in the Department of Defense Healthcare System. Participants are comprised of active duty service members, dependents of service members, and retirees from active service along with their dependents. As such, the participants in ICHP include both genders with a broad spectrum of ages, races and ethnicities. Some patients entering ICHP have diagnosed coronary heart disease but the large majority is seeking to reduce CV risk factors.

Upon entry to ICHP, patients meet with a nurse practitioner (NP) to undergo a CV-focused history and physical examination and submit a cardiac-relevant laboratory panel of tests. Based on this baseline assessment, patients are categorized as low, intermediate, or high risk for CVD by the Framingham Risk Score, the most widely used CVD risk estimator. Family history of premature CVD was collected and defined as a parent or sibling who had a CV event before the age of 55 in men and 65 in women. Patients also complete a series of validated questionnaires to determine their individual pattern of lifestyle behaviors.

Specific questionnaires focus on the domains of the program and are administered at baseline and at program completion, at 12 months: nutrition (Rate Your Plate), exercise (minutes of continuous exercise per week), stress (Perceived Stress Scale), and sleep (Pittsburgh Sleep Quality Index, Fatigue Visual-Analog Scale). A CV-relevant Self-Efficacy Questionnaire is administered at baseline and after an empowerment workshop, at 8 weeks from baseline (See Table 1).

Table 1. Time Points for Program Milestones

	Time 1 (Baseline)	Time 2 (8 Weeks)	Time 3 (12 Months)
Framingham Risk Calculation	X		
Self-Efficacy Questionnaire	X	X	
Empowerment Intervention	X	X	
Rate-Your-Plate Nutrition Score	X		X
Exercise Minutes per Week	X		X
Perceived Stress Scale	X		X
Pittsburgh Sleep Quality Index	X		X
Fatigue Score	X		X

Self-Efficacy Questionnaire: Self-efficacy was measured with the adapted diabetes mellitus type 2 self-efficacy scale. Since most self-management tasks apply generally to chronic diseases as a whole, this scale was used to measure the level of confidence people have about their ability to perform the self-management tasks necessary to reduce vascular risk. The 9-item questionnaire is scored on a 5-point Likert scale, with a higher self-efficacy score corresponding with better self-efficacy. Reliability of the questionnaire was tested with a Cronbach's alpha of 0.69 (Sol, van der Graaf, van der Bijl, Goessens, & Visseren, 2006).

Framingham Risk Score: Cardiovascular disease risk was calculated using the standard FRS Hard Coronary Heart Disease (10-Year Risk) (National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2002). The tool, which currently forms the foundation of current primary prevention guidelines, uses age, gender, total cholesterol, HDL cholesterol, smoking history and blood pressure (BP) to calculate the risk of coronary heart disease outcomes (MI and coronary death) over the subsequent 10 years. Scores are categorized as low ($\leq$10), medium (11-19) and high ($\geq$20).

Rate Your Plate (RYP): This ICHP-modified 26-item nutrition questionnaire, originally developed in 1983 by the Pawtucket Heart Health Program (PHHP), consists of questions focusing on foods that contribute the most fat, saturated fat, and cholesterol to the American nutrition. In a calibration study, the RYP was compared with the widely used Willet food frequency questionnaire (FFQ). When the RYP was administered prior to the Willet FFQ, Pearson product-moment correlations ranged between -0.45 and -0.65 on fat variables and nutrition cholesterol ($p < .001$ for all correlations), thus having the capacity to quantitatively reflect intake of fat and saturated fat (Gans, Hixson, Eaton, & Lasater, 2000), (Gans et al., 1993). The RYP individual score can indicate whether the participant's typical eating pattern is relatively high or low in fat, saturated fat and cholesterol. This questionnaire has been modified over the years to reflect changing national nutrition recommendations, fat-reduced foods now available in the marketplace, eating out and consideration of trans fatty acids in recommendations for spreads and cooking oils including the ICHP modifications to reflect use of beer, wine, alcohol, soda and other sugary drinks. Scores range from 26-78 with 26-42 reflecting least "heart healthy"; 43-60 middle ground, and; 61-78 most "heart healthy".

Perceived Stress Scale: PSS-14 developed in 1983 (Cohen et al., 1983) is one of the most widely accepted of measurements of stress (Cohen, Kamarck, & Mermelstein, 1983). Validation studies show that the PSS-14 has an internal consistency reliability of 0.85 by Cronbach alpha and a test-retest reliability of 0.85. This 14-item questionnaire asks the patient how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0 to 56 with higher scores correlating with higher stress. The PSS is designed for use with community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Scores in the low 20's reveal moderate stress levels while scores approaching 30 are substantial and concerning.

Pittsburgh Sleep Quality Index: PSQI is a self-rated questionnaire used to assess sleep quality and disturbances over a 1-month time interval (Buysse, Reynolds 3rd, Monk, Berman, & Kupfer, 1989). Nineteen individual items generate seven component scores whose sum yields one global score with a range of 0 to 21. The psychometric and clinical properties of the PSQI suggest its utility both in clinical practice and research

activities. A PSQI score greater than 5 has a diagnostic sensitivity of 89.6% and specificity of 86.5% (kappa = 0.75, $p \leq 0.001$). Essentially, a global score of greater than 5 indicates a poor sleeper. Sleep perturbations can be categorized by scores as follows: 0 to 5 is a good sleep score; 6 to 10 shows mild sleep difficulty; 11 to 15 moderate sleep difficulty, and 16 to 21 severe sleeping difficulty.

Fatigue Visual Analog Scale: This scale is borrowed from the Stanford Patient Education Research Center where it was tested on 122 patients, with mean value of 4.89 and standard deviations of 2.71 (Stanford Patient Education Research Center). This fatigue scale asks patients to express their experience of fatigue from 0 to 10 for the previous 2 week period. Patients who circle 5 to 6 express mild fatigue, 7 to 8 moderate fatigue, and 9 to 10 severe fatigue.

Empowerment Intervention: Empowerment strategies for patients are comprised of a comprehensive risk assessment report with detailed lifestyle recommendations for optimizing risk reduction generated by the NP. After two initial NP appointments which allow patients to problem-solve barriers to lifestyle change, clarify goals and identify motivations, patients attend a multi-disciplinary educational workshop. The workshop is comprised of an interactive healthy food demonstration and stress management experience and focus on the impact of actionable behaviors on health. For the remainder of the program and after the workshop, patients receive ongoing motivational advice by health coaches in order to achieve healthy goals in the program's domains as established by the NP in the first two weeks.

Statistics: Sample size calculation indicated that a sample of 100 participants would provide a stabilized and generalizable set of data. Plans were made to enroll approximately 115 patients to allow for a 15 percent rate of drop-out for failure to complete surveys properly. Data are presented as means with standard deviations or proportions. A median score was used to split patient Self-Efficacy Scores into two groups of nearly equal size allowing comparisons between high and low scoring groups. Demographic and survey health variables were compared using chi-square test for categorical variables, and Student's t-test for continuous variables. Pearson product-moment correlation coefficients were used to measure correlations because the data sets were normally distributed as seen on inspection of population histograms. All tests assumed $p < 0.05$ as statistical significance.

3. Results

The study population is comprised of 119 consecutive graduates of the ICHP CV program. The demographic variables of the group are provided in Table 2. The population is generally late middle aged, evenly split between men and women, representative of a variety of races, predominantly married and living in a family unit. Patients had an average of 2.5 CVD risk factors each, thus comorbid illnesses were common. Of the 119 patients, 9 (8%) were diagnosed with coronary heart disease, 67 (66%) with dyslipidemia, 61 (51%) with hypertension, 39 (33%) with obstructive sleep apnea, 32 (27%) with depression, 9 (8%) with diabetes, and 25 (21%) with pre-diabetes. Family history of premature CVD was reported by 53 patients (45%) in the total group. There were 20 (17%) patients who served as caregivers for other family members at home.

Table 2. Demographic variables for participants in the ICHP Registry

		All Patients (n = 119)	Low Self-Efficacy* (n = 59)	High Self-Efficacy** (n = 60)	p value***
Age (Years ± SD)		56.5 ± 13.1	55.2 ± 13.7	57.8 ± 12.6	0.28
Sex# men (%)		57 (48)	28 (47)	29 (48)	0.92
Race	White	85	41	44	0.85
	Black	21	12	9	
	Hispanic	5	3	2	
	Asian	2	1	1	
	Other	6	2	4	
Marital Status	Single	10	8	2	0.18
	Married	94	45	49	
	Divorced	11	5	6	
	Separated	4	1	3	

Number of Children	One	25	14	11	0.54
	Two	13	6	7	
	Three	39	18	21	
	Four or More	8	2	6	

*Low Self-Efficacy is defined as the group scoring below the median score of 36 points.

** High Self-Efficacy is defined as the group scoring at or above the median score of 36 points.

***p value denotes statistical difference between Low and High Self-Efficacy Groups by t-test for age and by chi square test for other variables.

Inspection of a histogram of the self-efficacy scores revealed that these were normally distributed. The median score (36 points) of the self-efficacy questionnaire measured at entry to ICHP was used to divide the participants into high (n=60) and low scorers (n=59). Demographic variables were not different for high and low self-efficacy subgroups (See Table 2). Framingham risk scores were calculated for each patient showing that nearly one third of patients were at intermediate or high risk for a CVD event over 10 years. Low self-efficacy patients were at higher CVD risk than high self-efficacy patients by Framingham estimation (See Table 3).

Table 3. At baseline, low Self-Efficacy correlates with higher CVD risk

	Medium or High Risk **by Framingham**	**p value***
Low Self-Efficacy (**n=59**)	36%	0.04
High Self-Efficacy (**n=60**)	22%	

*Chi square analysis shows a significant difference between groups.

At baseline, the low self-efficacy group entered the ICHP program with lower scores for a healthy nutrition, less exercise minutes per week, higher levels of perceived stress, poorer sleep quality and greater fatigue (See Table 4).

Table 4. Change in Outcomes from Baseline to Completion According to Self-Efficacy Score

		All Patients (n = 119)	**Low Self-Efficacy* (n = 59)**	**High Self-Efficacy** (n = 60)**	**p value*** **
Self-Efficacy (of 45 points)	Baseline	34.5 ± 6.5	29.1 ± 0.8	39.9 ± 3.0	NA
	Completion	40.3 ± 4.2	38.2 ± 4.6	42.4 ± 2.2	< 0.001
	Change	5.8, p<0.001	9.1, p<0.001	2.5, p<0.001	< 0.001
Nutrition (of 78 points)	Baseline	61.7 ± 8.3	58.9 ± 4.1	64.5 ± 7.4	< 0.001
	Completion	67.1 ± 6.0	65.7 ± 6.6	68.6 ± 5.0	0.008
	Change	5.4, p<0.001	6.8, p<0.001	4.1, p<0.001	0.01
Exercise (minutes per week)	Baseline	156 ± 125	110 ± 87	201 ± 141	< 0.001
	Completion	220 ± 163	186 ± 157	253 ± 163	0.02
	Change	64, p<0.001	76, p=0.002	52, p=0.06	0.16
Perceived Stress (of 56 points)	Baseline	20.1 ± 9.1	22.0 ± 8.5	18.3 ± 9.3	0.02
	Completion	17.2 ± 8.6	18.3 ± 8.7	16.1 ± 8.4	0.16
	Change	2.9, p=0.01	3.7, p=0.02	2.2, p=0.18	0.18
Sleep Quality (of 21 points)	Baseline	7.1 ± 3.9	7.9 ± 4.3	6.2 ± 3.2	0.02
	Completion	4.7 ± 3.5	5.3 ± 4.1	4.1 ± 2.7	0.06
	Change	2.4, p<0.001	2.6, p=0.001	2.1, p<0.001	0.53

Fatigue (of 10 points)	Baseline	4.3 ± 2.5	5.0 ± 2.4	3.6 ± 2.3	0.001
	Completion	3.0 ± 2.2	3.2 ± 2.3	2.9 ± 2.1	0.39
	Change	1.3, p<0.001	1.8, p<0.001	0.7, p=0.07	0.01

*Low Self-Efficacy is defined as the group scoring below the median score of 36 points.

** High Self-Efficacy is defined as the group scoring at or above the median score of 36 points.

***p value denotes statistical difference by t-test between Low and High Self-Efficacy Groups.

These findings were corroborated with Pearson r product-moment correlations which showed a strong correlation of nutrition scores and moderately strong correlations of exercise minutes, lower stress scores, and better sleep quality with total self-efficacy scores (See Table 5).

Table 5. At baseline, improvements in Self-Efficacy Score correlate with improvements in health indices.

	Nutrition Score	**Exercise Minutes**	**Stress Levels**	**Sleep Quality**
Total SE Score	0.47 (p<0.001)	0.37 (p<0.001)	0.30 (p=0.03)	0.36 (p<0.001)

The Pearson r coefficients show a strong correlation between baseline self-efficacy score and nutrition score and moderately strong correlations for exercise, stress and sleep.

In response to the empowerment intervention of the ICHP program, 98 of the total 119 patients (82%) showed gains in self-efficacy with an average improvement of 7.2 ± 4.4 points; 11 (9%) showed no change; and 10 (8%) decreased their self-efficacy an average of 2.0 ± 1.2 points. In the group of 59 patients with low self-efficacy at program entry, 58 (98%) showed improvements averaging 9.4 ± 4.4 points in self-efficacy and only one (2%) decreased self-efficacy by 2.0 points.

Among all 119 participants, only three (3%) were active tobacco smokers, each reporting current smoking of 2 cigarettes per day. Nineteen other patients were former smokers, having an average 1 pack per day history of smoking, and having quit an average of 26 years prior. Since tobacco use occurred so infrequently in the study population, further analysis of tobacco use was not performed.

4. Discussion

The salient finding of the current study is that a boost to self-efficacy early in a lifestyle intervention program produces substantial improvements in behavioral outcomes. The overwhelming majority of patients responded with improved self-efficacy scores. Self-efficacy scores increased irrespective of baseline self-efficacy.

Though patients in both the low and the high self-efficacy groups showed improvements in self-efficacy and behavioral survey scores, the largest gains in self-efficacy occurred in patients who ranked in the lower half for self-efficacy at baseline. This lower self-efficacy group also demonstrated behavioral improvements that erased differences between the high and low self-efficacy groups or at least provided a substantial "catch-up" such that the scores on completion of the program were approaching or better than baseline scores for the high self-efficacy group.

Our findings agree with prior reports that self-efficacy scores at baseline correlate with the cardiovascular risk profile. Indeed in our population, patients with low self-efficacy scores were found to have a higher predicted cardiovascular risk by Framingham Risk Score in addition to less healthy cardiovascular behaviors (Table 3). Given the burden of CV risk and comorbid illness in the low self-efficacy group it is critical to provide self-care behavioral tools to overcome lifestyle behavioral change barriers.

Prior studies assessing the impact of self-efficacy on adherence to behavior change have frequently been limited to a single behavioral dimension such as nutrition (Sharp & Salyer, 2012), (Timlin, Shores, & Reicks, 2002), (Nothwehr, 2008), (Cha, 2014) or exercise (Slovinec D'Angelo, Pelletier, Reid, & Huta, 2014), (Schwarzer, Luszczynska, Ziegelmann, Scholz, & Lippke, 2008). Investigations evaluating the impact of a self-efficacy intervention on multiple behaviors have had mixed results. One randomized controlled trial reported positive effects on nutrition and exercise but not on smoking and alcohol intake (Sol et al., 2008). Another prospective cohort study showed the beneficial effect of increased self-efficacy on nutrition, exercise and stress management

(Clark & Dodge, 1999). The present prospective cohort study shows positive effects of improved self-efficacy on nutrition, exercise, and stress management behaviors but extends the positive effects to sleep improvement as well.

Employing an empowerment intervention early in the sequence of events in a heart-healthy program provides a mechanism for increased patient self-efficacy. Our findings validate numerous studies showing that interventions that aim to empower patients are valuable in promoting patient well-being, decision-making and self-management of chronic disease (Aujoulat, Marcolongo, Bonadiman, & Deccache, 2008).

The results of the current study appear to be generalizable to other locations and institutions. This group of patients at risk for CVD mirrors the population at large with regard to demographic profile and comorbid illnesses. The intervention that was provided does not require special equipment or resources and is therefore scalable and could be duplicated in other centers targeting CVD prevention.

The current study has limitations. Because the intervention aimed at multiple dimensions of healthy CV behaviors, it is not possible to determine which aspects of the empowerment intervention were most effective. Likewise, with the current study design it is not possible to determine whether or not there was a synergistic impact from improvement in one behavior that helped stimulate improvements in other behaviors. A second limitation is the use of measurement tools that rely on self-report. While these tools are validated instruments to measure the behaviors for which they were targeted, the use of objective measures would give more robust information and therefore be more convincing. Unfortunately, objective measures are complex (nutrition measures), unwieldy (actigraphy for exercise and sleep), or do not readily exist (stress management).

In summary, the results of this study support the idea that a lifestyle behavioral change program aimed at providing an early boost to self-efficacy is feasible and can yield positive results. These findings are particularly significant in high-risk patients who are vulnerable to CVD and may be in a position to make critical behavioral lifestyle modifications to lower their risk of overt disease. Further study is warranted to measure the impact that such behavior changes have on prevention of CVD events.

Acknowledgements

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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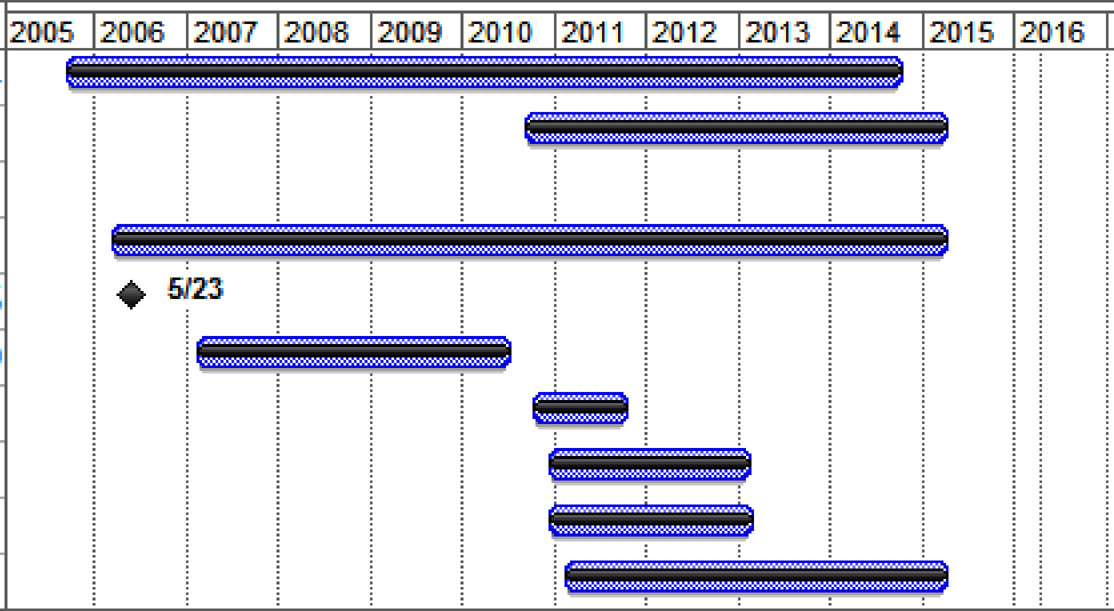
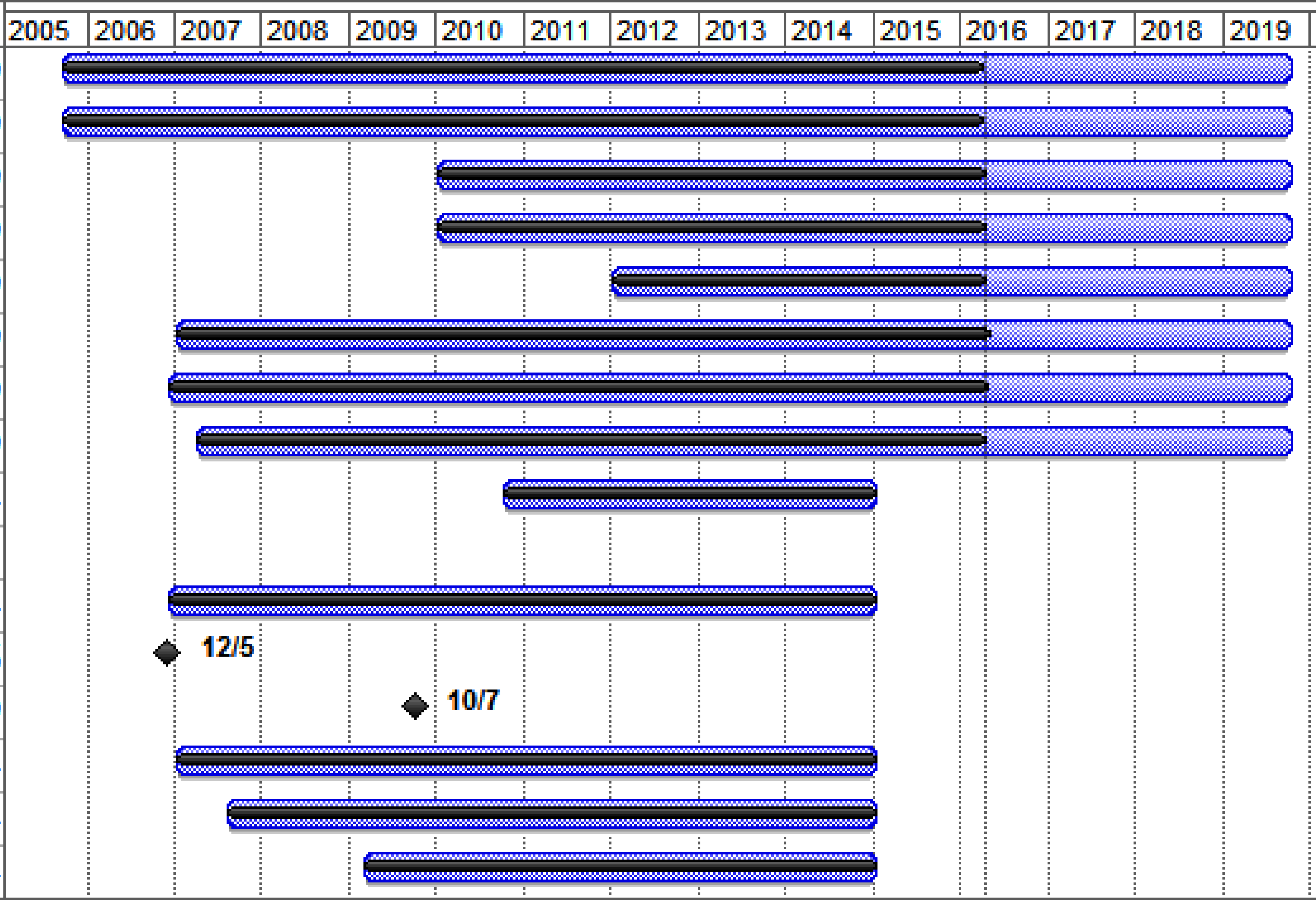
APPENDIX B
GANTT CHARTS

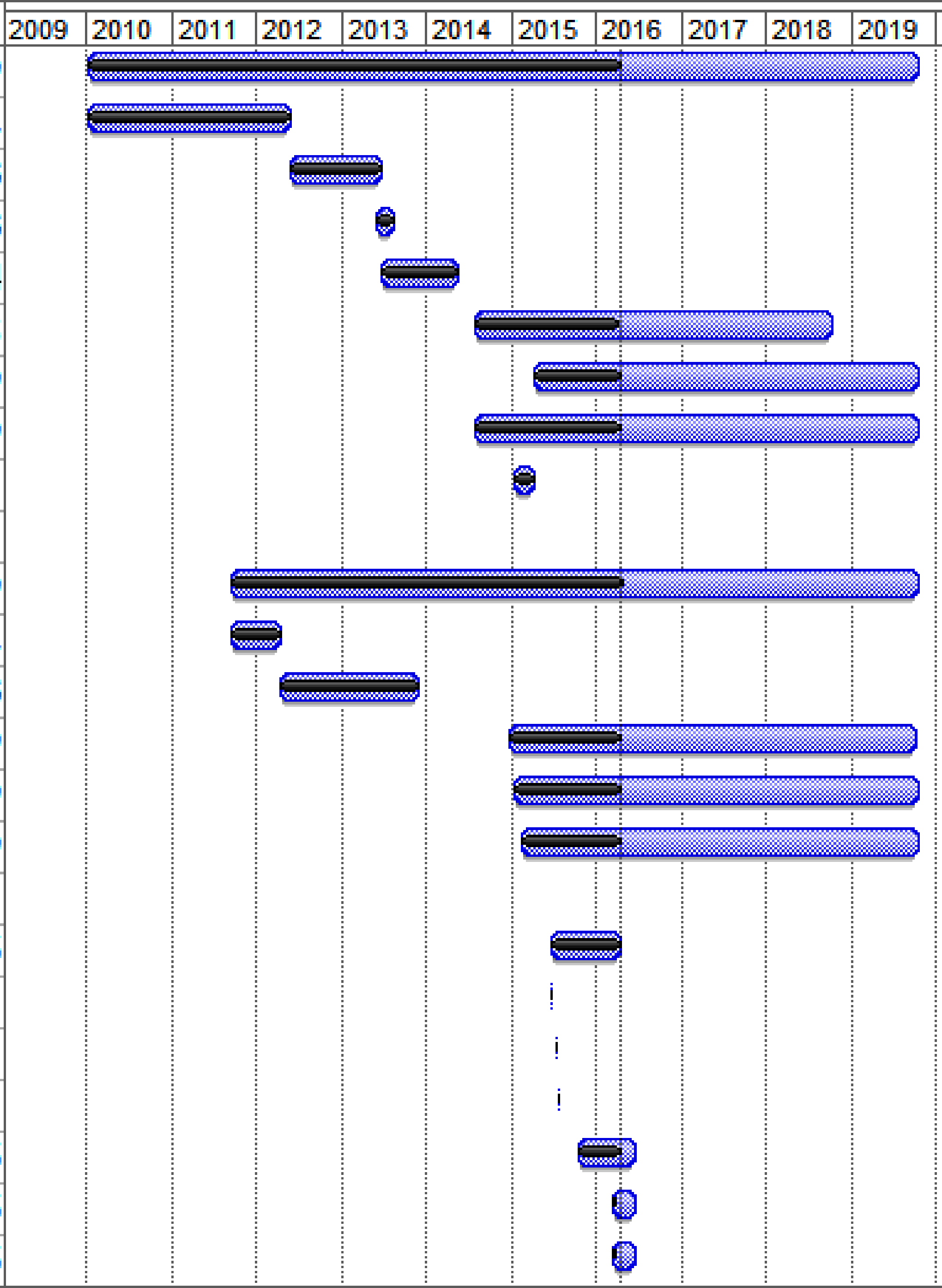
ID	ℹ	Task Name	Start	Finish
1	✓	Task #1: BATTLE trial - COMPLETED	Thu 9/1/05	Mon 9/29/14
2	✓	Presentations and manuscripts	Wed 9/1/10	Tue 3/31/15
3				
4	✓	Task #2: CADRe 5Yr Follow-up - COMPLETED	Wed 3/1/06	Tue 3/31/15
5	✓	IRB protocol approval	Tue 5/23/06	Tue 5/23/06
6	✓	Participant enrollment/Data collection	Fri 2/2/07	Wed 6/30/10
7	✓	Data reconciliation	Fri 10/1/10	Fri 9/30/11
8	✓	Conduct analysis	Wed 12/1/10	Thu 1/31/13
9	✓	Publication plan	Wed 12/1/10	Fri 2/15/13
10	✓	Presentations and manuscripts	Tue 2/1/11	Tue 3/31/15



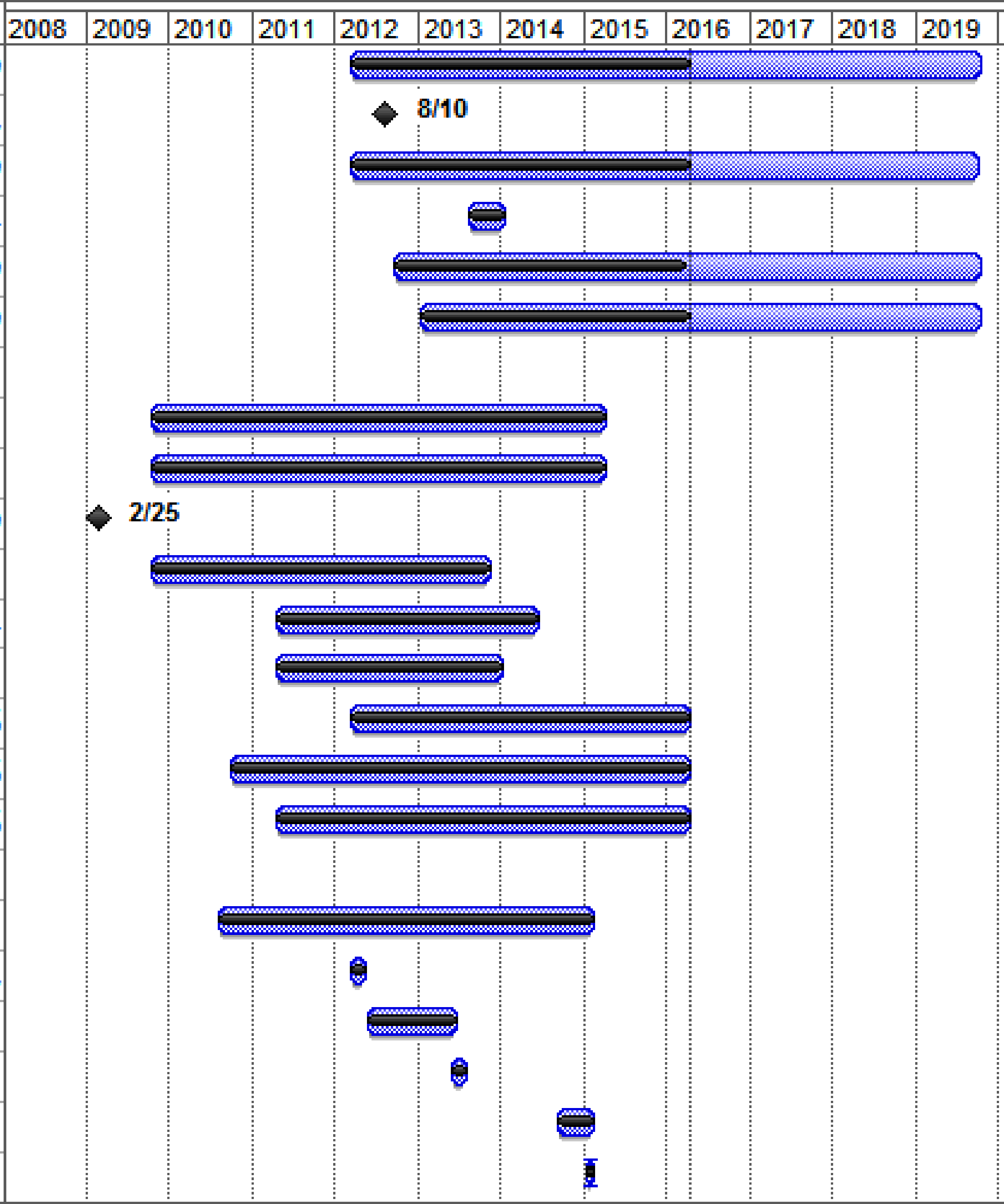
ID		Task Name	Start	Finish
1		Task #3: Continue CHP	Thu 9/1/05	Sun 9/29/19
2		Enrollment/Data collection	Thu 9/1/05	Sun 9/29/19
3		Advance data modeling	Fri 1/1/10	Sun 9/29/19
4		Outreach	Fri 1/1/10	Sun 9/29/19
5		Ultra personal empowering	Mon 1/2/12	Sun 9/29/19
6		Outcomes analysis	Mon 1/1/07	Sun 9/29/19
7		Target subgroup populations	Fri 12/1/06	Sun 9/29/19
8		Presentations/manuscripts	Mon 4/2/07	Sun 9/29/19
9	✓	Upgrade database	Fri 10/1/10	Wed 12/31/14
10				
11	✓	#3.1: Validate CV risk-CLOSED	Tue 12/5/06	Wed 12/31/14
12	✓	IRB protocol approval	Tue 12/5/06	Tue 12/5/06
13	✓	Continuing review approved	Wed 10/7/09	Wed 10/7/09
14	✓	Data collection	Mon 1/1/07	Mon 12/29/14
15	✓	Conduct analysis	Wed 8/1/07	Mon 12/29/14
16	✓	Presentations/manuscripts	Mon 3/2/09	Mon 12/29/14



ID		Task Name	Start	Finish
1		Subtask #3.2: Initiate ZENITH trial - CLOSED	Fri 1/1/10	Sun 9/29/19
2	✓	Protocol development	Fri 1/1/10	Wed 5/9/12
3	✓	Protocol approval WRNMMC/MRMC	Thu 5/10/12	Mon 6/10/13
4	✓	Protocol approval at WMC/MRMC	Fri 5/17/13	Wed 7/31/13
6	✓	Study execution planning	Fri 6/14/13	Wed 4/30/14
7		Recruitment/enrollment/data collection	Tue 7/15/14	Fri 9/28/18
8		Conduct analysis	Wed 4/1/15	Sun 9/29/19
9		Biomolecular studies	Tue 7/15/14	Sun 9/29/19
10	✓	Publication plan	Fri 1/2/15	Sun 3/29/15
11				
12		Subtask #3.3: CHP Prospective Registry	Thu 9/1/11	Sun 9/29/19
13	✓	Protocol development/submission	Thu 9/1/11	Fri 3/30/12
14	✓	Protocol approvals (WRNMMC/MRMC)	Mon 4/2/12	Wed 11/13/13
15		Recruitment/enrollment/data collection	Mon 12/15/14	Fri 9/27/19
16		Data reconciliation/analysis	Fri 1/2/15	Sun 9/29/19
17		Manuscript preparation	Mon 2/2/15	Sun 9/29/19
18				
19	✓	Subtask #3.4 CHP CBT-Insomnia Study (Phase 1)	Mon 6/8/15	Thu 3/31/16
20	✓	WRNMMC IRB approval	Mon 6/8/15	Mon 6/8/15
21	✓	USUHS Concurrence	Tue 6/30/15	Tue 6/30/15
22	✓	USAMRMR HRPO approval	Fri 7/10/15	Fri 7/10/15
23		Recruitment/enrollment/data collection	Thu 10/1/15	Tue 5/31/16
24		Data analysis	Tue 3/1/16	Tue 5/31/16
25		Manuscript preparation/presentation	Tue 3/1/16	Tue 5/31/16



ID		Task Name	Start	Finish
1		Subtask #3.4: CV Risk in Tramatic Amputations	Thu 3/1/12	Sun 9/29/19
2	✓	Protocol approval	Fri 8/10/12	Fri 8/10/12
3		Recruitment/enrollment/data collection	Thu 3/1/12	Fri 9/27/19
4	✓	Protocol modification for genomics/analytes	Thu 8/1/13	Thu 1/2/14
5		Data analysis	Mon 9/3/12	Sun 9/29/19
6		Presentations and manuscripts	Tue 1/1/13	Sun 9/29/19
7				
8	✓	Task #4: Global Profiling/CRC Completion	Thu 10/1/09	Sun 3/29/15
9	✓	Followup data analysis/publication	Thu 10/1/09	Sun 3/29/15
10	✓	Enroll program participants	Wed 2/25/09	Wed 2/25/09
11	✓	Manuscript on gene expression	Thu 10/1/09	Thu 10/31/13
12	✓	TaqMan SNP analysis	Thu 4/14/11	Fri 5/30/14
13	✓	Metabolite profiling analysis	Thu 4/14/11	Tue 12/31/13
14	✓	Assimilation of PET/CT data	Thu 3/1/12	Tue 3/29/16
15	✓	Conduct molecular analysis	Wed 9/15/10	Tue 3/29/16
16	✓	Presentations & publications	Thu 4/14/11	Tue 3/29/16
17				
18	✓	Task #6: Nat History Pre-Diabetes-CLOSED	Mon 8/2/10	Thu 2/5/15
19	✓	Protocol development/submission	Thu 3/1/12	Mon 5/7/12
20	✓	Protocol approval at WRNMMC/MRMC	Thu 5/10/12	Tue 6/4/13
21	✓	Protocol approval at WMC/MRMC	Fri 5/17/13	Wed 7/24/13
22	✓	Study execution and planning	Mon 9/1/14	Thu 2/5/15
23	✓	Recruitment/enrollment/data collection	Thu 1/1/15	Thu 2/5/15



ID	ℹ	Task Name	Start	Finish
1	✓	Task #7: Morbid Obesity - COMPLETED	Fri 1/13/06	Sun 3/29/15
2	✓	Protocol approved at WMC	Fri 1/13/06	Fri 1/13/06
3	✓	Protocol approved at TATRC	Fri 6/15/12	Fri 6/15/12
4	✓	Enroll patients	Mon 7/24/06	Fri 8/30/13
5	✓	Obtain blood and tissue samples	Mon 7/24/06	Mon 3/31/14
6	✓	Conduct molecular analysis	Mon 10/1/12	Sun 3/29/15
7	✓	Presentations and manuscripts	Tue 1/1/13	Sun 3/29/15
8				
9	✓	Task #8: Global Long-term - COMPLETED	Fri 8/17/12	Sun 3/29/15
10	✓	Protocol approved at WMC	Fri 8/17/12	Fri 8/17/12
11	✓	Protocol approved at TATRC	Thu 5/2/13	Thu 5/2/13
12	✓	Enroll patients	Sat 6/1/13	Tue 4/1/14
13	✓	Obtain blood samples and data	Sat 6/1/13	Tue 4/1/14
14	✓	Conduct molecular analysis	Tue 10/1/13	Sun 3/29/15
15	✓	Presentations and manuscripts	Wed 1/1/14	Sun 3/29/15